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ANAEROBIC DECOMPOSITION OF WHEAT STRAW BY THERMOPHILES AND THE QUANTITY OF GAS PRODUCED[1]

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INTRODUCTION

This contribution is a continuation of the series (*3*, *4*, *5*,)[3] on the changes in fibrous farm wastes caused by anaerobic microbial fermentation and the significance of these changes in the possible utilization of such materials for the production of fuel gas, pulp, and fertilizer. The first paper (*3*) dealt specifically with the quantity and quality of fuel gas that could be produced from these various wastes, particularly cornstalks, wheat straw, artichoke tops, and seed flax straw. It was shown that the rate of gas production by the anaerobic fermentation of farm wastes was greater at 50° to 55° C. than at 28° to 30°, although the total amount of gas produced was about the same. The degree of fineness of the material and possibly its internal structure played an important part in its susceptibility to decomposition. Periodic chemical analyses of the residues were not made, since the main objective was to determine how much utilizable gas could be produced from a unit amount of waste material.

The next logical step was to ascertain at what rate the individual components of the wastes were decomposed, because this information should be of value in planning the preliminary treatment of these wastes in the preparation of pulp, in retting, and in composting. The authors had found that at 28° to 30° C. (*4*) and at 50° to 55° (*5*) cornstalk flour was fermented more rapidly than chopped cornstalks and produced more gas in a given unit of time; in cornstalk flour the losses of cellulose and pentosans were considerably greater, and the loss of lignin less, than in the chopped cornstalks. The loss of lignin in the cornstalk flour was much less than the loss of either the cellulose or the pentosans.

The study reported in this paper was undertaken to determine the decomposition of the various constituents of wheat straw by a thermophilic anaerobic fermentation and the quantity of gas produced.

PROCEDURE

The test materials were prepared by grinding wheat straw in a hammer mill. The material ground fine enough to pass a circular

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[3] Italic numbers in parentheses refer to Literature Cited, p. 144.

opening 1 mm. in diameter is called straw flour. The term "chopped straw" is used to designate the material composed wholly of coarse particles about one-fourth of an inch in length.

The thermophilic methane-producing seed was originally developed from sewage sludge, as described in a previous publication (*3*). Before it was used it was passed through a screen with circular openings 1 mm. in diameter. All solids remaining on the screen were discarded. The seed was analyzed, and sufficient urea was added (0.90 gm. per liter) to bring the ammonia content of the mixture to from 500 to 800 parts per million.

The fermentation apparatus consisted of a 2-quart mason jar into the neck of which was inserted a rubber stopper provided with three tubes for (1) the removal of samples for pH determination, (2) the addition of chemicals, and (3) the collection of gas. The rubber stopper was held in position by means of the metallic mason-jar lid, the center of which was cut out to permit protrusion of the tube outlets. Details of construction of the fermentation, gas-collection, and gas-measurement apparatus have been described by the authors in previous publications (*3*, *4*).

The fermentation mixtures consisted of 30 gm. of the wheat straw and 1 liter of the seed. At the beginning of the experiment, hydrated lime was added (equivalent to 1 gm. of CaO per liter of mixture) to adjust the mixture to the desired pH. The mixtures were incubated at 50° to 55° C. for 20 days, and analyses were made periodically.

At the end of each fermentation period the mixtures were removed from the incubator and treated as follows: All the straw-flour mixtures were evaporated to dryness on the steam hot plate, ground to pass a 30-mesh screen, and analyzed. In the chopped-wheat-straw mixture it was necessary to separate the seed from the straw in order to ascertain the actual loss of constituents of the straw. The seed was therefore removed with a screen having round holes 1 mm. in diameter. The straw fraction retained on the screen was washed several times with distilled water, a total of 1,000 cc. being used. The washed straw was then dried on the steam hot plate, ground to pass a 30-mesh screen, and analyzed. The sludge fraction (the portion that passed through the screen) and the washings from the straw fraction were combined, evaporated to dryness, ground to pass a 30-mesh screen, and analyzed.

The following determinations were made on the dried residues: (1) Total solids; (2) volatile solids (loss on ignition at about 600° C. for 1 hour) by the American Public Health Association method (*1*); (3) pentosans by the Association of Official Agricultural Chemists' method (*2*); (4) cellulose by the Norman and Jenkins' method (*6*) (with corrections made for ash and pentosans); (5) alpha cellulose as outlined by Schorger (*9*); and (6) lignin by the Norman and Jenkins' method (*7*) with the substitution of a 2-percent-acid hydrolysis for 2 hours for the 5-percent hydrolysis normally used. Duplicate lignin samples were taken. One was ashed, and the other was analyzed for Kjeldahl nitrogen by the American Public Health Association method (*1*). A correction for ash was made in calculating the results, but no correction for nitrogen was made, inasmuch as Norman and Jenkins (*8*) have shown that the usual protein factor is not reliable.

Gas was collected from the following fermentations: (1) Straw flour plus active methane-producing seed; (2) chopped straw plus seed;

(3) seed (control); (4) straw flour plus water (control); and (5) chopped straw plus water (control). The gas produced each day was measured, and its composition was determined periodically. The pH was ascertained daily.

DISCUSSION OF RESULTS

DECOMPOSITION OF CONSTITUENTS

The quantities of the various constituents in the straw flour, in the chopped straw, and in the seed, before fermentation, are shown in table 1. Table 2 shows the decomposition of constituents in the seed control. Most of the changes (losses or increases) noted in the constituents in the seed control are very small and probably are within the experimental error of the methods of analysis and of the procedure used in this study. These small changes are not considered significant.

TABLE 1.—*Constituents in wheat-straw-flour and chopped-wheat-straw mixtures before fermentation*

Constituents	Wheat-straw-flour mixture			Chopped-wheat-straw mixture		
	Seed	Wheat-straw flour	Total	Seed	Chopped wheat straw	Total
Total solids.....grams..	15.5	28.4	[1] 45.7	15.5	28.4	[1] 45.7
Volatile solids.....do....	10.4	26.4	[2] 37.7	10.4	27.0	[2] 38.3
Pentosans.....do....	.5	8.3	8.8	.5	8.5	9.0
Crude cellulose.....do....	.6	15.5	16.1	.6	15.1	15.7
Pentosans in the cellulose.....do....	.1	5.2	5.3	.1	4.7	4.8
Cellulose (corrected) [3].....do....	.5	10.3	10.8	.5	10.4	10.9
Alpha cellulose.....do....	.....	10.2	.....	.....	9.8	.....
Lignin.....do....	5.6	4.3	9.9	5.6	4.4	10.0
Kjeldahl nitrogen in the lignin..percent...	2.2	.5	.....	2.2	1.0	.....

[1] Includes 0.90 gm. of urea and 1.0 gm. of CaO.
[2] Includes 0.90 gm. of urea.
[3] Corrected for ash and pentosans.

Data on the break-down of the various constituents in the straw-flour fermentation are recorded in table 3, and similar data for the chopped straw are recorded in table 4. The results are presented graphically in figure 1. The values shown in table 4 were obtained from tables 5 and 6 by taking the sum of the constituents of the straw fraction and of the sludge fraction of the chopped-straw mixture. The percentage losses of the various constituents, listed in tables 3 and 4, were computed after corrections were made for losses in the seed (table 2).

Throughout the straw-flour fermentation, the percentage losses of cellulose and pentosans, which were nearly the same, were rather high. The cellulose was attacked as readily as the pentosans, and as the losses were high the residue would probably be of little value for making paper pulp. The behavior of these constituents was different from that in the fermentation of cornstalks (*5*), where the loss of pentosans during the earlier stages of the fermentation was usually greater than that of cellulose.

In the chopped-straw fermentations, the percentage losses of cellulose and pentosans were about the same during the entire fermentation period. In this material, also, the high loss of cellulose would be unfavorable for a fermentation paper-pulping process.

TABLE 2.—*Decomposition of constituents in the seed control and quantity of gas produced after fermentation for various periods*

Time fermented (days)	Quantity present								Quantity decomposed [1]						Gas produced [2]		
	Total solids	Volatile solids	Pentosans	Crude cellulose	Pentosans in the cellulose	Cellulose (corrected) [3]	Lignin	Kjeldahl nitrogen in the lignin	Total solids	Volatile solids	Pentosans	Pentosans in the cellulose	Cellulose (corrected) [3]	Lignin	Daily	Cumulative total	Per gram of volatile solids lost
	Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Cubic centimeter*	*Cubic centimeter*	*Cubic centimeter*
0	15.5	10.4	0.5	0.6	0.1	0.5	5.6	2.2									
1	15.3	10.3	.5	.6	.1	.5	5.5		0.9	1.4	0.0	0	0.0	1.8	45	45	322
2	15.2	10.2	.5	.6	.1	.6	5.5		2.1	2.2	.0	0	+20.0	1.8	0	45	196
3	15.0	10.0	.5	.7	.1	.6	5.4		3.2	3.4	.0	0	+20.0	3.6	0	45	128
5	14.6	9.8	.5	.7	.1	.6	5.3		6.0	5.4	.0	0	+20.0	5.4	0	45	80
10	13.7	9.3	.4	.7	.1	.7	5.1	1.3	11.7	10.2	20.0	0	+40.0	8.9	0	45	42
20	13.1	8.8	.4	1.0	.1	.9	4.9	1.6	15.5	15.1	20.0	0	+80.0	12.5	0	45	29

[1] Plus sign indicates a gain. [2] Corrected to 30 inches of mercury and 60° F. [3] Corrected for ash and pentosans.

TABLE 3.—*Decomposition of constituents in the wheat-straw-flour mixture (straw flour + seed) and quantity of gas produced after fermentation for various periods*

Time fermented (days)	Quantity in mixture									Decomposition of constituents in straw [1]						Gas produced [2] per gram of—			Total gas [2] produced
	Total solids	Volatile solids	Pentosans	Crude cellulose	Pentosans in the cellulose	Cellulose (corrected) [3]	Alpha cellulose	Lignin	Kjeldahl nitrogen in the lignin	Total solids	Volatile solids	Pentosans	Pentosans in the cellulose	Cellulose (corrected) [3]	Lignin	Wheat-straw flour	Volatile solids in straw	Volatile solids lost	
	Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Cubic centimeters*	*Cubic centimeters*	*Cubic centimeters*	*Cubic centimeters*
0 [4]	45.7	37.7	8.8	16.1	5.3	10.9	10.2	9.9											
1	34.6	29.4	6.6	11.2	3.4	7.8	7.8	8.9	1.9	38.4	31.1	26.5	36.5	30.1	20.9	30.5	32.8	105	865
2	33.1	27.5	6.1	10.5	3.2	7.3	7.2	8.7	2.3	43.3	37.9	32.5	40.4	35.0	25.6	87.9	94.5	250	2,495
3	32.5	26.3	5.4	8.1	2.4	5.6	5.6	8.4	2.4	44.7	41.7	41.0	55.8	51.5	30.2	140.0	150.6	361	3,975
5	31.1	25.1	4.5	7.5	2.2	5.4		8.7	2.4	48.2	45.5	51.8	59.6	53.4	20.9	188.4	202.7	446	5,350
10	26.6	19.8	2.3	3.2	.9	2.3		8.3	2.6	60.9	63.6	77.1	84.6	83.5	25.6	320.6	344.9	542	9,105
20	23.1	17.3	1.8	2.5	.7	1.8		7.6	1.9	71.1	71.2	83.1	88.5	88.3	37.2	375.7	404.2	568	10,670

[1] All figures in this section were computed after making corrections for losses in the seed (table 2).
[2] Corrected to 30 inches of mercury and 60° F.
[3] Corrected for ash and pentosans.
[4] For composition of original mixture see table 1.

[1] All figures in this section were computed after making corrections for losses in the seed (table 2).
[2] Corrected to 30 inches of mercury and 60° F.
[3] Corrected for ash and pentosans.
[4] For composition of original mixture see table 1.

TABLE 4.—*Decomposition of constituents in the chopped-wheat-straw mixture (straw+seed) and quantity of gas produced after fermentation for various periods*

Time fermented (days)	Quantity in mixture									Decomposition [1] of constituents in straw [2]						Gas produced [3] per gram of—			Total gas [3] produced
	Total solids	Volatile solids	Pentosans	Crude cellulose	Pentosans in the cellulose	Cellulose (corrected) [4]	Alpha cellulose	Lignin	Kjeldahl nitrogen in the lignin	Total solids	Volatile solids	Pentosans	Pentosans in the cellulose	Cellulose (corrected) [4]	Lignin	Chopped wheat straw	Volatile solids in straw	Volatile solids lost	
	Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Cubic centimeters*	*Cubic centimeters*	*Cubic centimeters*	*Cubic centimeters*
0 [5]	45.7	38.3	9.0	15.7	4.8	10.9	9.8	10.0											
1	41.2	35.5	8.4	15.8	4.7	11.0		9.4		15.5	10.0	7.1	0.2	+1.0	11.4	23.9	25.2	252	680
2	38.6	32.8	6.8	13.5	3.9	9.6		9.6		24.3	19.6	25.9	19.1	12.5	6.8	71.7	75.4	384	2,035
3	38.8	31.9	6.3	12.0	3.4	8.6		9.8		22.9	22.2	31.8	29.8	22.1	.0	116.7	122.8	553	3,315
5	36.6	29.9	5.5	10.4	3.5	6.9		10.0		29.2	28.9	41.2	27.7	38.5	+6.8	178.2	187.4	649	5,060
10	32.9	26.2	4.7	7.7	2.1	5.6		9.6		39.1	40.7	49.4	57.4	51.0	+2.3	287.3	302.2	742	8,160
20	28.0	21.1	2.6	5.1	1.6	3.6		8.8		54.2	57.8	74.1	68.1	70.2	11.4	372.5	391.9	678	10,580

[1] Plus sign indicates a gain.
[2] All figures in this section were computed after making corrections for losses in the seed (table 2).
[3] Corrected to 30 inches of mercury and 60° F.
[4] Corrected for ash and pentosans.
[5] For composition of original mixture see table 1.

Cellulose and pentosans were decomposed much more rapidly in the straw flour that in the chopped straw throughout the entire fermentation period. The difference in break-down between the flour and the chopped material is interesting because practically no difference was noted in the total volume of gas produced from the flour and from the chopped straw (table 9). During the straw-flour fermentation some depressing action may have occurred that retarded the rate of gas production from the intermediate complexes formed during the break-down of the cellulose and the pentosans. This retarded rate of gas production might have been due to a difference in the nature of the break-down in the flour, but more likely it was due to the formation of an intermediate complex highly resistant to the production of

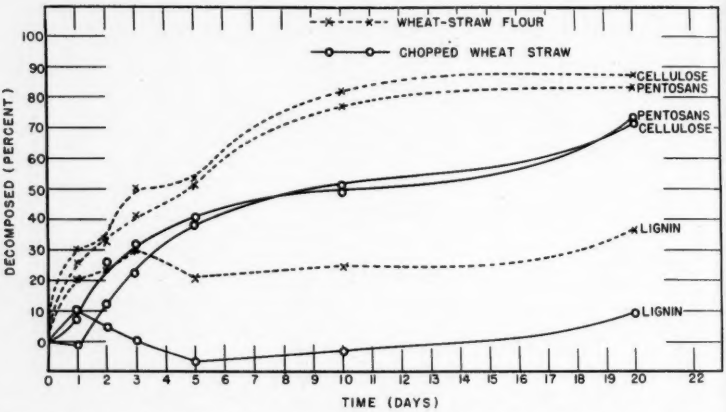
WHEAT-STRAW FLOUR
CHOPPED WHEAT STRAW
CELLULOSE
PENTOSANS
PENTOSANS
CELLULOSE
LIGNIN
LIGNIN
DECOMPOSED (PERCENT)
TIME (DAYS)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 22
0 10 20 30 40 50 60 70 80 90 100

FIGURE 1.—Decomposition of the constituents of wheat straw in an active methane-producing seed at 50° to 55° C.

gas. However, further study is necessary to ascertain the reasons for this behavior.

The percentage losses of the pentosans associated with the cellulose, shown in tables 3 and 4 and graphically in figure 2, were practically the same as the corresponding percentage losses of cellulose in both the straw-flour and the chopped-straw fermentations.

Determination of alpha-cellulose was made when sufficient residue was available. These values, given in tables 3 and 4, are compared graphically with the Norman and Jenkins' cellulose (corrected for ash and pentosans) in figure 3. In both fermentations the values for alpha-cellulose agree fairly well with the corrected Norman and Jenkins' values for cellulose, and for practical purposes the corrected cellulose values may be considered as alpha-cellulose values in analyzing this type of material. This application is of considerable importance in the analysis of sludge samples in which the cellulose content is so low that sufficient material is not available for the actual alpha-cellulose determination.

In the chopped straw, the decomposition of lignin was very small. The lignin decomposed much more rapidly in straw flour, but even here it was more resistant to attack than were the cellulose and pentosans. In fact, during the earlier stages of the straw-flour fer-

mentation little decomposition was noted. Later, when most of the cellulose and pentosans had been decomposed, lignin was broken down at an appreciable rate.

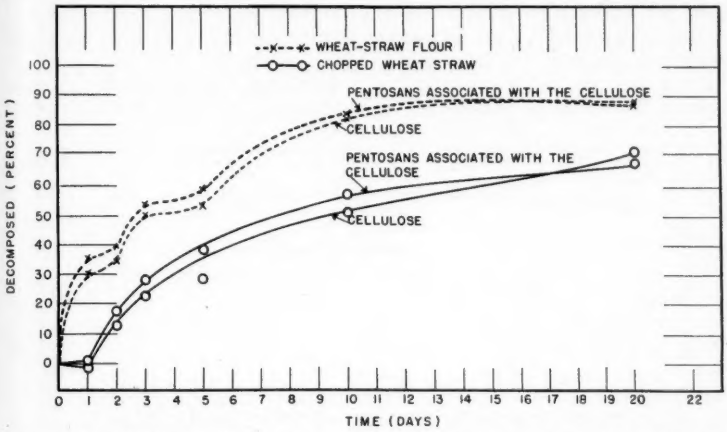

FIGURE 2.—Decomposition of the cellulose and pentosans associated with the cellulose in wheat straw in an active methane-producing seed at 50° to 55° C.

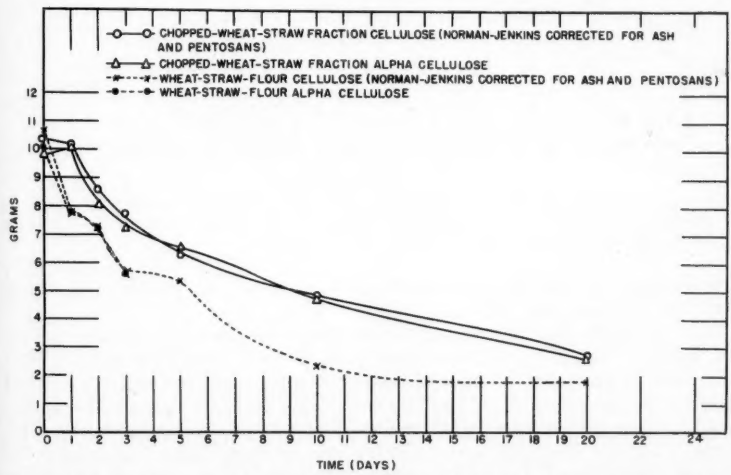
CHOPPED-WHEAT-STRAW FRACTION CELLULOSE (NORMAN-JENKINS CORRECTED FOR ASH AND PENTOSANS)
CHOPPED-WHEAT-STRAW FRACTION ALPHA CELLULOSE
WHEAT-STRAW-FLOUR CELLULOSE (NORMAN-JENKINS CORRECTED FOR ASH AND PENTOSANS)
WHEAT-STRAW-FLOUR ALPHA CELLULOSE
GRAMS
TIME (DAYS)

FIGURE 3.—Alpha cellulose and Norman and Jenkins' cellulose (corrected for ash and pentosans) in wheat straw in an active methane-producing seed at 50° to 55° C.

The behavior of lignin in both the straw-flour and the chopped-straw fermentation was rather striking. In both, decomposition of lignin was noted in the earlier stages of the fermentation, followed by an apparent increase and finally by a gradual decrease. In many cases this increase in lignin content was accompanied by an increase in Kjeldahl nitrogen in the lignin residue. This is shown graphically

in figure 4. Probably lignonitrogenous complexes were formed during the fermentation, especially during the earlier stages, and when the actual break-down was slight, these complexes increased the weight of apparent lignin (residue after the 72-percent-acid treatment). The real break-downs of lignin was probably greater than that shown by the anaylsis. Other investigators, Waksman and Iyer (*10*) and Norman and Jenkins (*8*) have noted this, and it has also been noted in earlier work in this laboratory (*4*). However, no definite conclusions can be drawn as to the actual decomposition of lignin because of the

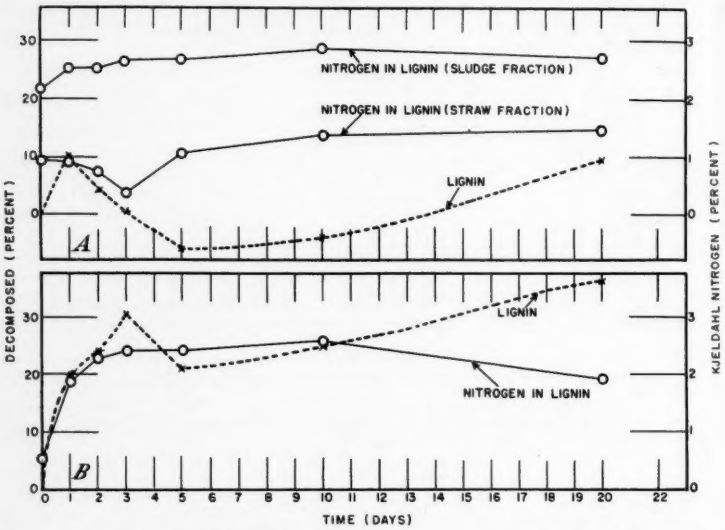

FIGURE 4.—Decomposition of lignin and the percentage of Kjeldahl nitrogen in the lignin residue in (*A*) chopped wheat straw and (*B*) wheat-straw flour in an active methane-producing seed at 50° to 55° C.

inadequacy of the present methods of analysis, as has been pointed out by Norman and Jenkins (*7*, *8*).

In the straw-flour fermentation, in which appreciable decomposition of lignin occurred during the latter part of the fermentation, a distinct decrease was noted in the Kjeldahl nitrogen content of the lignin residue after 20 days' fermentation. This indicated that the lignoprotein complexes were undergoing decomposition. Apparently this attack on the lingoprotein complex was greater than that observed by Waksman and Iyer (*10*), who considered these complexes to be very resistant to microbial attack.

The break-down of the various constituents in the straw fraction from the chopped-straw fermentation is recorded in table 5, and that of the sludge fraction (material passing through a 1-mm. screen plus washings from the straw) in table 6. The results are presented graphically in figure 5.

TABLE 5.—*Quantity of constituents in the straw fraction of the chopped-wheat-straw mixture and the percentage removed after fermentation for various periods*

Time fermented (days)	Quantity present									Percentage removed					
	Total solids	Volatile solids	Pentosans	Crude cellulose	Pentosans in the cellulose	Cellulose[1] (corrected)	Alpha cellulose	Lignin	Kieldahl nitrogen in the lignin	Total solids	Volatile solids	Pentosans	Pentosans in the cellulose	Cellulose[1] (corrected)	Lignin
	Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
0[2]	28.4	27.0	8.5	15.1	4.7	10.4	9.8	4.4	1.0						
1	24.2	23.3	7.7	14.7	4.5	10.2	10.1	4.2	.9	14.8	13.7	9.4	4.3	1.9	4.5
2	21.1	20.3	6.1	12.4	3.8	8.6	8.0	3.8	.8	25.7	24.8	28.2	19.1	17.3	13.6
3	19.7	18.8	5.5	11.0	3.2	7.8	7.2	3.7	.4	30.6	30.4	35.3	31.9	25.0	15.9
5	17.2	16.5	4.9	9.6	3.3	6.2	6.6	3.6	1.1	39.4	38.9	42.4	29.8	40.4	18.2
10	13.4	12.7	4.2	6.8	2.0	4.9	4.7	3.2	1.4	52.8	53.0	50.6	57.4	52.9	27.3
20	8.2	7.7	2.0	4.0	1.1	2.8	2.6	2.1	1.5	71.1	71.5	76.5	76.6	73.1	52.3

[1] Corrected for ash and pentosans.
[2] For composition of original mixture see table 1.

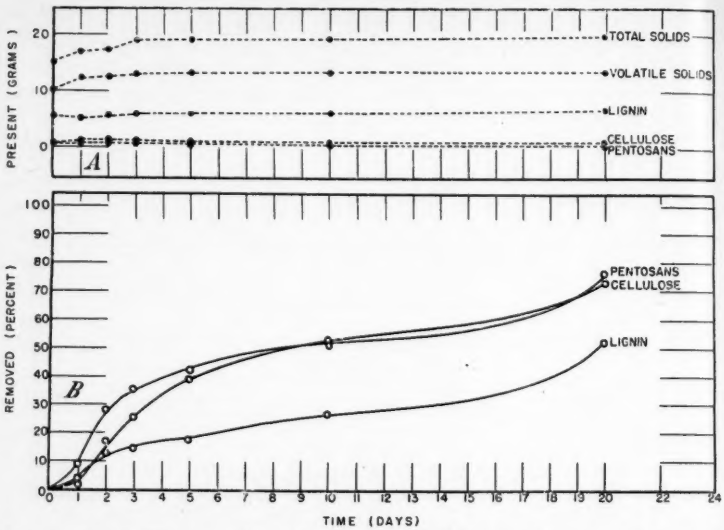

FIGURE 5.—*A*, The quantities of pentosans, cellulose, and lignin, together with the solids, present in the sludge fraction; *B*, percentage of pentosans, cellulose, and lignin removed from the straw fraction of chopped wheat straw in an active methane-producing seed at 50° to 55° C.

TABLE 6.—*Constituents in the sludge fraction of the chopped-wheat-straw mixture after fermentation for various periods*

Time fermented (days)	Total solids	Volatile solids	Pentosans	Crude cellulose	Pentosans in the cellulose	Cellulose [1] (corrected)	Lignin	Kjeldahl nitrogen in the lignin
	Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Percent*
0 [2]	15.5	10.4	0.5	0.6	0.1	0.5	5.6	2.2
1	17.0	12.2	.7	1.1	.2	.9	5.2	2.5
2	17.5	12.5	.7	1.1	.1	1.0	5.8	2.5
3	19.2	13.1	.8	1.1	.2	.8	6.0	2.7
5	19.3	13.3	.6	.8	.1	.7	6.4	2.7
10	19.5	13.4	.6	.8	.1	.7	6.4	2.9
20	19.8	13.5	.6	1.2	.5	.7	6.7	2.7

[1] Corrected for ash and pentosans.
[2] For composition of original mixture see table 1.

The break-down of the pentosans and cellulose in the straw fraction occurred at about the same rate as in the mixture, indicating that these constituents had not accumulated in the sludge fraction. They probably had been broken down into intermediate complexes as rapidly as they separated from the coarse particles of the straw. However, this was apparently not true for lignin. Analysis of the straw fraction showed that the lignin had decreased about as rapidly as the cellulose and pentosans, but when the sludge fraction was analyzed, it was noted that a large part of the lignin had not been decomposed but had accumulated in the sludge. In the mixture early loss of lignin was noted, followed by an apparent increase and finally by a decrease. No corresponding increase in lignin was observed in the straw fraction (fig. 5, *B*). Apparently most of the lignonitrogenous complexes were formed in the sludge fraction. This

accounts for the apparent increase in lignin (fig. 1). There was, however, a slight increase in Kjeldahl nitrogen in the lignin residue from the straw fraction (fig. 4).

After the third day, the percentage losses of pentosans and cellulose from the straw fraction were practically the same, indicating that a prefermentation period would be of little value in the manufacture of paper pulp from wheat straw. In breaking down the pentosans sufficiently, considerable cellulose would also be broken down. However, a short fermentation period might have value in making board pulp.

Little break-down of the constituents occurred in the seed (control) and in the straw flour and the chopped straw fermented in water (controls), as shown by the data in tables 2 and 7.

TABLE 7.—*Decomposition of constituents in wheat straw fermented in water at 50° to 55° C. for 20 days*

Constituents	Wheat-straw flour—			Chopped wheat straw—		
	Before fermentation	After fermentation	Decomposed[1]	Before fermentation	After fermentation	Decomposed[1]
Total solids.....grams	28.4	27.5	0.9	28.4	27.0	1.4
Volatile solids.....do	26.4	25.6	.8	27.0	25.9	1.1
Pentosans.....do	8.3	7.7	.6	8.5	8.3	.2
Crude cellulose.....do	15.5	15.0	.5	15.1	15.1	.0
Pentosans in the cellulose.....do	5.2	4.9	.3	4.7	4.7	.0
Cellulose (corrected)[2].....do	10.3	10.1	.2	10.4	10.4	.0
Alpha cellulose.....do	10.2	9.8	.4	9.8	10.1	+.3
Lignin.....do	4.3	4.5	+.2	4.4	4.4	.0
Kjeldahl nitrogen in the lignin..percent	.5	.8	.....	1.0	.2	.....

[1] + indicates a gain.
[2] Corrected for ash and pentosans.

The use of this anaerobic type of fermentation should be of value in composting straws. The rate of break-down of the constituents is much higher by this method than that reported in most of the literature on composting, and a gas is produced that could be used either for heating or for the production of power. However, no work has been done to determine the value of the residue as a fertilizer.

PRODUCTION OF GAS

The volumes of gas produced, corrected to 30 inches (760 mm.) of mercury and 60° F. (15.6° C.), are recorded in table 8 and shown graphically in figure 6. The total volume of gas produced per gram of volatile solids present appears to be appreciably lower than the corresponding values for many of the other agricultural wastes, especially cornstalks (*3*).

No significant differences were noted in the volumes of gas produced from the straw flour and from the chopped straw throughout the entire fermentation period. The size of the particles of the material seemed to have little effect on the rate of gas production, indicating that no appreciable time would be saved by grinding wheat straw. This behavior is different from that found in studies on cornstalk fermentation (*4*, *5*), where the fine material, the flour, fermented and produced gas much more rapidly than the coarse material, the chopped cornstalks. Since wheat straw and cornstalks are similar chemically, containing approximately the same quantities of cellulose,

pentosans, and lignin, one might expect these two materials to react very much alike when fermented. They did not, however, and some factor or factors other than gross chemical composition probably influenced the fermentation.

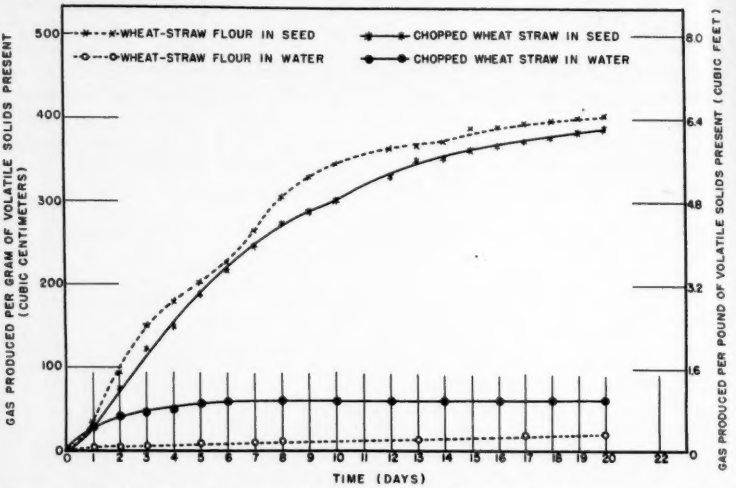

FIGURE 6.—Quantity of gas produced from wheat straw in an active methane-producing seed and in water at 50° to 55° C.

TABLE 8.—*Gas produced from the wheat-straw mixtures and seed control fermented for various periods at 50° to 55° C.*[1]

Time fermented (days)	Cumulative quantity of gas produced per gram of volatile solids in the straw and pH values of the mixture[2]								pH of seed control
	Wheat-straw flour				Chopped straw				
	In active seed[3,4]		In tap water		In active seed[4,5]		In tap water		
	Gas produced	pH of mixture	Gas produced	pH of mixture	Gas produced	pH of mixture	Gas produced	pH of mixture	
	Cc.		*Cc.*		*Cc.*		*Cc.*		
1	32.8	6.8	3.2	6.0	25.2	6.8	27.2	6.3	7.2
2	94.5	6.8	4.8	6.0	75.4	6.8	40.7	6.1	7.6
3	150.6	7.0	6.4	6.0	122.8	6.9	47.6	6.1	7.7
4	178.2	6.9	6.4	6.0	153.7	6.9	51.1	6.1	8.0
5	202.7	7.0	9.5	6.0	187.4	6.9	58.0	6.3	7.7
6	224.7	7.0	9.5	6.0	217.6	7.0	61.2	6.1	8.0
7	264.6	7.0	11.1	6.0	246.3	6.9	61.2	6.1	8.2
8	305.5	7.2	12.7	6.3	273.0	6.9	64.6	6.3	8.2
9	326.5	7.0	12.7	6.1	286.8	6.9	64.6	6.1	8.1
10	344.9	7.1	12.7	6.1	302.2	6.9	64.6	6.3	8.1
12	359.0	7.2	12.7	6.1	331.2	7.0	64.6	6.4	8.1
13	375.7	.....	15.9	.....	351.3	.....	64.6	.....	.....
14	382.7	7.2	15.9	6.1	354.6	7.2	64.6	6.5	8.1
15	389.4	.....	15.9	.....	361.3	.....	64.6	.....	.....
16	392.8	7.3	15.9	6.3	367.8	7.3	64.6	6.3	8.2
17	396.0	.....	19.0	.....	374.5	.....	64.6	.....	.....
18	399.7	.....	19.0	.....	381.3	.....	64.6	.....	.....
19	401.4	.....	19.0	.....	388.1	.....	64.6	.....	.....
20	404.3	7.3	19.0	6.3	391.9	7.3	64.6	6.5	8.1

[1] Corrected to 30 inches of mercury and 60° F.

[2] Computations were made after corrections were made for gas produced in the seed control. The total gas produced by the seed control was only 45 cc. and this was produced on the first day.

[3] Average gas composition: CO_2, 29.4 percent; H_2, 2.4 percent; CH_4, 56.5 percent.

[4] Corrected for 45 cc. of gas produced by seed control.

[5] Average gas composition: CO^2, 27.6 percent; H_2, 2.5 percent; CH_4, 58.2 percent.

The quantities of gas (*4*) that would be available if gas were produced from all the cellulose and pentosans broken down are shown in table 9. These calculated values and the actual quantities of gas produced are compared graphically in figure 7 for the straw flour

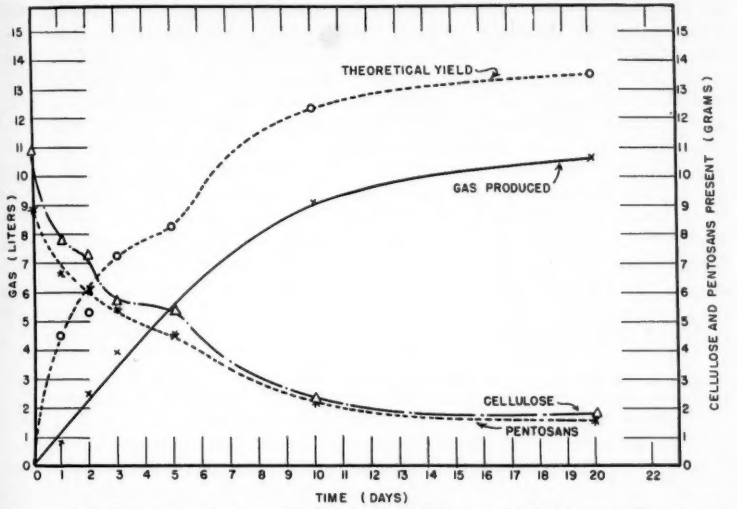

FIGURE 7.—Quantity of gas produced from 28.35 gm. of wheat-straw flour in an active methane-producing seed at 50° to 55° C., the theoretical yield based on the quantities of cellulose and pentosans decomposed, and the quantities of cellulose and pentosans left after the fermentation.

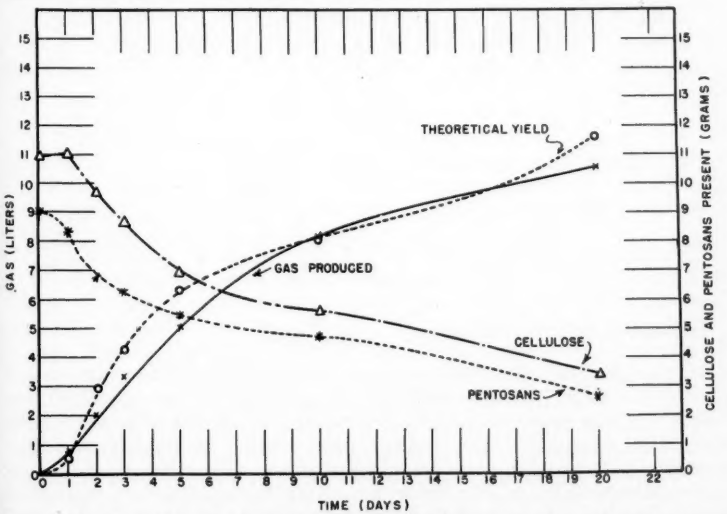

FIGURE 8.—Quantity of gas produced from 28.37 gm. of chopped wheat straw in an active methane-producing seed at 50° to 55° C., the theoretical yield based on the quantities of cellulose and pentosans decomposed, and the quantities of cellulose and pentosans left after the fermentation.

and in figure 8 for the chopped straw. The quantities of cellulose and of pentosans remaining are also shown in these figures.

In the straw-flour fermentation the pentosans and the cellulose broken down could have produced a quantity of gas considerably larger than that actually produced. As has already been noted, the total quantity of gas produced was considerably less than that produced from an equal quantity of cornstalk flour (*4*, *5*). Inasmuch as these two waste materials are very similar chemically, it is unlikely that the variation in gas production by the two materials was due to a difference in gross chemical composition. The difference might be attributed to their physical structure or the configuration of their components, but this probably was not the sole reason, since the actual break-down of pentosans and of cellulose in the wheat-straw flour tends to approach the corresponding break-down in the cornstalk flour. Some intermediate reaction in the wheat-straw-flour fermentation may have exerted a depressing action on the production of gas by the complexes resulting from the cellulose and pentosan break-down. Further investigation is necessary to ascertain the cause of this behavior.

TABLE 9.—*Quantity of gas produced from wheat-straw flour and from chopped wheat straw and the theoretical yield based on the quantities of pentosans and cellulose decomposed after fermentation for various periods*

Time fermented (days)	Wheat-straw-flour mixture [1]					Chopped wheat-straw mixture [1]				
			Gas [2]					Gas [2]		
	Pentosans decomposed	Cellulose decomposed	Actual yield	Theoretical yield based on quantity of pentosans and cellulose decomposed	Ratio of theoretical yield to actual yield	Pentosans decomposed	Cellulose decomposed	Actual yield	Theoretical yield based on quantity of pentosans and cellulose decomposed	Ratio of theoretical yield to actual yield
	Gm.	*Gm.*	*Cc.*	*Cc.*		*Gm.*	*Gm.*	*Cc.*	*Cc.*	
1	2.2	3.1	865	4,445	5.1	0.6	+0.1	680	535	0.8
2	2.7	3.6	2,495	5,285	2.1	2.2	1.3	2,035	2,965	1.5
3	3.4	5.2	3,975	7,270	1.8	2.7	2.3	3,315	4,240	1.3
5	4.3	5.5	5,350	8,215	1.5	3.5	4.0	5,060	6,340	1.3
10	6.6	8.5	9,105	12,630	1.4	4.3	5.3	8,160	8,035	1.0
20	7.1	9.1	10,670	13,510	1.3	6.4	7.3	10,580	11,610	1.1

[1] 30 gm.
[2] Corrected to 30 inches of mercury and 60° F.

In the chopped-straw fermentation practically all the cellulose and pentosans decomposed could be accounted for by the gas produced (fig. 8). This was in marked contrast to the findings in the straw-flour fermentations. As already pointed out, the total quantity of gas produced by the chopped straw was less than that produced by chopped cornstalks (*4*, *5*). However, as all the cellulose and pentosans lost from the chopped straw could be accounted for by the gas produced, no lag period (time from decomposition to production of gas) occurred, as was the case with the chopped cornstalks. Probably no

depressing action, such as was noted in the straw-flour fermentation, was exerted on the production of gas by the intermediate complexes resulting from the break-down of the cellulose and pentosans. Very likely the principal reason for the difference in the quantities of gas produced by the chopped cornstalks and the chopped straw was a difference in the physical structure or the configuration of their components. The influence of physical structure would probably be more pronounced in the chopped material than in the flour.

SUMMARY AND CONCLUSIONS

Wheat-straw flour and chopped wheat straw were fermented for 20 days at 50° to 55° C. The pH was determined periodically. Daily measurements of the gas produced were made.

Percentage decomposition of cellulose and pentosans was high in the straw-flour fermentation. Little difference in the percentage decomposition of cellulose and of pentosans was noted, probably indicating that no selective fermentation took place. Lignin appeared to be the most resistant of these three major plant constituents, although appreciable break-down of lignin was observed during the later stages of the fermentation.

The decomposition of cellulose and of pentosans was much smaller in the chopped straw than in the straw flour. The break-down of pentosans and of cellulose was practically the same, and no selective fermentative action was noted. Only slight decomposition of lignin was noted throughout this fermentation.

In both fermentations, the formation of lignonitrogenous complexes was indicated. The actual break-down of lignin, therefore, was probably greater than that shown by the analysis. In the straw-flour fermentation, in which appreciable decomposition of lignin occurred during the latter part of the fermentation, a distinct decrease was noted in the Kjeldahl nitrogen content of the lignin residue after 20 days' fermentation, indicating that the lignonitrogenous complexes were being appreciably attacked.

The values for alpha cellulose corresponded closely to the values for the Norman and Jenkins' cellulose corrected for ash and pentosans. Apparently, these values may be used interchangeably for material of this type. This application would be of value in analyses in which an adequate quantity of sample for the actual alpha cellulose determination is not available.

Approximately the same volumes of gas were obtained from the straw flour (10,670 cc.) and the chopped straw (10,580 cc.). This was interesting in view of the fact that considerably greater quantities of the straw-flour constituents were broken down during the fermentation. In the straw-flour fermentation a depressing action probably was exerted on the production of gas by the complexes resulting from the break-down of the cellulose and the pentosans. If all the material broken down had produced gas, much greater quantities of gas would have been produced.

The quantity of gas produced per unit weight (gram of volatile solids) of raw material was considerably less for the wheat straw (flour, 404 cc.; chopped, 392 cc.) than was previously noted for cornstalks (flour, 543 cc.; chopped, 401 cc.).

Possibilities for the utilization of a fermentation such as is reported herein in the manufacture of paper pulp from wheat straw do not seem

promising. The loss of cellulose is too high and too nearly the same as the loss of pentosans to make this a promising paper-pulping procedure. However, the use of a short fermentation period may have some value in the making of board pulp.

This type of anaerobic fermentation may have some value in composting straws.

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EFFECT OF VARIOUS CONCENTRATIONS OF PAPAIN AND POTASSIUM IODATE ON THE LOAF VOLUME OF BREAD [1]

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INTRODUCTION

The mechanism of the action of chemical bread improvers has recently been the subject of wide discussion. Jørgensen (*9, 10, 11, 12, 13*),[2] and Balls and Hale (*2, 3*) have advanced the theory that the improvement of baking quality caused by oxidizing agents, such as potassium bromate and potassium iodate, is due to the fact that they inactivate the proteinases of flour, thereby inhibiting the action of these enzymes on the flour proteins. These writers also believe that the flour proteinases belong to the papain group.

This theory is supported by Flohil (*6*) and is opposed by Read and Haas (*16, 17, 18*), Carbonnelle (*5*), and Ritter (*19*). The arguments for and against the theory have been fully and ably summed up by the opposing sides in recent articles (*12, 13, 14, 18*).

In studies on the total and free amylase content of ungerminated seeds, baking tests were made on a sample of commercial wheat flour to which were added various concentrations of papain and potassium iodate. The results, reported here, may throw some added light on the disputed theories with regard to the mechanism of action of certain bread improvers.

PROCEDURE

The baking tests were conducted according to the standard procedure of the American Association of Cereal Chemists (*1*). The respective quantities of papain and potassium iodate specified in table 1 were added to the water (60 cc. per 100 gm. of flour) before it was mixed with the flour. The concentrations, percentage as well as molecular, are given on the basis of the water added. These tests were carried out in three series of bakings on different days. The respective concentrations of papain were the same in all three series. They ranged from 0.0005 to 0.01 percent, or from 0.3 to 6.0 mg. per 100 gm. of flour. Balls and Hale, who were the first to obtain a depression in the loaf volume of bread with papain, used 50 mg. per 315 gm. of flour or at the rate of 16 mg. per 100 gm. of flour. The concentrations of the potassium iodate solution, however, varied in the different series. They ranged from 0.00002 to 0.00008 molar or from 0.25 mg. to 1.0 mg. per 100 gm. of flour.

RESULTS

Table 1 and figures 1, 2, and 3 deal only with the loaf volume of the bread. The data in table 1 represent the products of the longitudinal and transverse circumferences of the loaves and serve only as a numerical expression of the relative volume of breads obtained with the vari-

[1] Received for publication August 16, 1941.
[2] Italic numbers in parentheses refer to Literature Cited, p. 151.

ous concentrations of papain with and without potassium iodate. The measurement data and the photographs are in good agreement.

In the first series of bakings (fig. 1), the concentration of the potassium iodate solution was 0.00008 molar (1 mg. in 100 gm. of

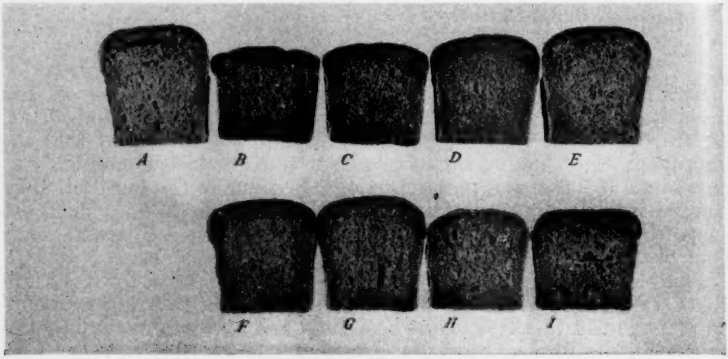

FIGURE 1.—Bread made with various concentrations of papain and potassium iodate. No papain or potassium iodate was added to *A* (control); to *B*, *C*, *D*, and *E*, papain was added in concentrations of 0.01, 0.005, 0.002, and 0.001 percent, respectively; to *F*, *G*, *H*, and *I*, an 0.00008 molar solution of potassium iodate (1 mg.) was added, together with 0.01, 0.005, 0.002, and 0.001 percent, respectively, of papain.

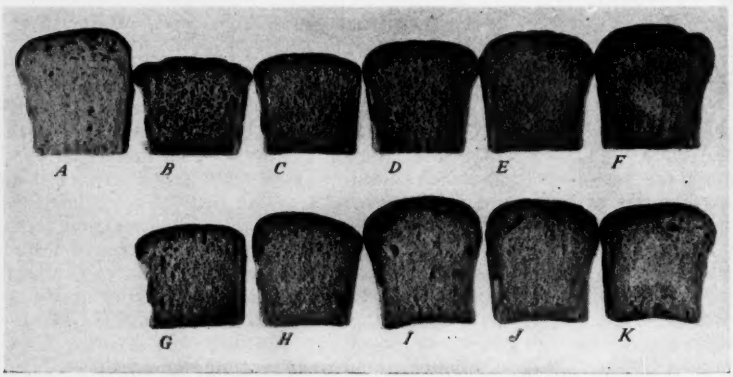

FIGURE 2.—Bread made with various concentrations of papain and potassium iodate. No papain or potassium iodate was added to *A* (control); to *B*, *C*, *D*, *E*, and *F*, papain was added in concentrations of 0.01, 0.005, 0.002, and 0.001, and 0.0005 percent, respectively; to *G*, *H*, *I*, *J*, and *K*, a 0.00004 molar solution of potassium iodate (0.05 mg.) was added, together with 0.01, 0.005, 0.002, 0.001, and 0.0005 percent, respectively, of papain.

flour). Without potassium iodate the loaf volume consistently decreased with the increased concentration of papain. The greatest depressions, caused by the concentrations of 0.01 and 0.005 percent of papain (6 and 3 mg. per 100 gm. of flour, respectively) were overcome to a large extent by the potassium iodate. But with the concentrations of 0.002 and 0.001 percent of papain (1.2 and 0.6 mg.

respectively per 100 gm. of flour), the loaf volume was less with potassium iodate than that of the bread made without potassium iodate. This would indicate that these low concentrations of papain were not sufficient to overcome the depressing effect of an 0.00008 molar potassium iodate solution. That some concentrations of potassium iodate added singly depress the loaf volume of bread is shown by the work of Jørgensen (*9*) and by unpublished results of this laboratory.

The concentrations of potassium iodate that evidently caused a depression in the loaf volume of bread in these experiments are much lower than those that caused a depression in Jørgensen's tests. The

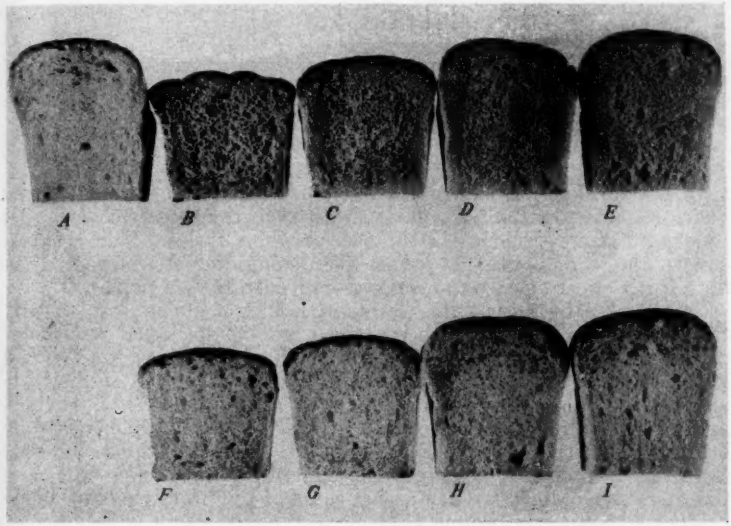

FIGURE 3.—Bread made with various concentrations of papain and potassium iodate. No papain or potassium iodate was added to *A* (control); to *B*, *C*, *D*, and *E*, papain was added in concentrations of 0.01, 0.005, 0.002, and 0.0005 percent, respectively; to *F*, *G*, *H*, and *I*, a 0.00002 molar solution of potassium iodate (0.25 mg.) was added, together with 0.01, 0.005, 0.002, and 0.0005 percent, respectively, of papain.

most likely explanation of the discrepancy is that different flours were used in the two sets of experiments.

In the second series the concentration of the potassium iodate solution was 0.00004 molar (0.5 mg. in 100 gm. of flour) or half that of the first series. Here, too, the results show that the depression in loaf volume was directly related to the concentration of papain. It is further shown that the concentration of 0.00004 molar of iodate was not so efficient in counteracting the depressing effect of 0.01 and 0.005 percent of papain (6 and 3 mg. per 100 gm. of flour) as the concentration of iodate used in the first series but it completely overcame the depressing effect of 0.002 percent of papain. These results indicate that the concentrations of 0.001 and 0.0005 percent of papain (0.6 and 0.3 mg. per 100 gm. of flour) were not strong enough to counteract the depressing effect of this concentration of potassium iodate.

TABLE 1.—*Loaf volume* [1] *of bread obtained with various concentratons of papain and potassium iodate*

Concentration of papain solution (percent) [2]	Series 1		Series 2		Series 3	
	Without potassium iodate	With an 0.00008 molar solution of potassium iodate [3]	Without potassium iodate	With a 0.00004 molar solution of potassium iodate [4]	Without potassium iodate	With a 0.00002 molar solution of potassium iodate [5]
None	199		209		216	
.01	156	189	160	164	165	164
.005	162	188	162	181	177	180
.002	177	158	182	212	197	206
.001	192	159	196	203		
.0005			205	190	211	224

[1] Longitudinal circumference multiplied by transverse circumference. The figures are numerical expressions of the relative volume of the loaves.
[2] On the basis of water added. To obtain percentages on the basis of flour, multiply by 0.6.
[3] 1.0 mg. in 100 gm. of flour.
[4] 0.5 mg. in 100 gm. of flour.
[5] 0.25 mg. in 100 gm. of flour.

In the third series, in which a 0.00002 molar (0.25 mg. in 100 gm. of flour) potassium iodate solution was used, the results consistently follow the same trend as in the two previous series. This weakest concentration of potassium iodate used completely counteracted the depressing effect of only the weakest concentration of papain.

The results of these three series of baking tests indicate clearly that potassium iodate, which is classed among the bread improvers, counteracts the depressing effect of an added proteinase on the loaf volume of bread. Flohil (*6*) was able to overcome the depression in loaf volume of bread caused by papain by adding potassium bromate, another bread improver. Read and Haas (*17*) admit that potassium bromate inhibits the proteolytic action of papain but doubt whether the wheat proteinases belong to the papain group. The evidence in the literature indicates that at least some of the proteinases of wheat belong to the papain group (*2*, *4*).

The results also show that under certain conditions the proteinases in flour may be beneficial, because they tend to overcome the depressing effects of oxidizing agents, such as potassium iodate. Possibly flour contains natural oxidizing substances, an excess of which would depress the loaf volume just as an excess of potassium iodate does. Under such conditions the addition of a proteinase might prove beneficial. Indeed, Read and Haas (*16*) found that the addition of proteinases had a beneficial effect on the baking qualities of flours that develop "bucky doughs."

DISCUSSION

In the baking tests reported here the loaf volume was depressed by the added papain in direct relation to its concentration, the added potassium iodate counteracted this effect, and an excess of iodate in turn depressed the loaf volume. Since it is generally accepted that the loaf volume of bread is associated with the strength of the gluten, the logical interpretation of these findings would be that the papain attacked the proteins, destroying their strength, and that potassium

iodate counteracted the effect of the papain. Consistent with this reasoning is the theory that potassium iodate, added in excess, depressed the loaf volume of the bread because it carried the inhibition of the proteinases too far, as a certain degree of proteolytic action is supposed to be necessary for the proper maturing of the gluten. This is essentially the theory advanced by Jørgensen (*9*, *13*) and by Balls and Hale (*2*, *3*).

Is a different interpretation of these facts possible?

It is known that papain liberates amylase in some ungerminated cereals (*7*). The tests reported here showed that papain added to the flour in the concentrations used in the baking tests also liberates amylase (table 2). Even the lowest concentrations of 0.0005 percent of papain had a pronounced effect in increasing the diastatic power of the flour. Potassium iodate in the concentration that counteracted the effect of papain and depressed the loaf volume of the bread in the baking tests (table 1) also depressed the diastatic activity of the flour (table 3). The extractions of the amylase in the last two experiments (tables 2 and 3) were carried out at about 70° F., according to the approximate procedure of the British Institute of Brewing (*8*). The effect of papain on the liberation of the flour amylase and the hydrolizing power of the latter, as well as the inhibiting effect of the potassium iodate, would be expected to be stronger at the temperature of dough ripening and of the first stages of baking (before the bread reaches the temperature of inactivation of the enzymes), than at 70° F. Therefore, while it is realized that the diastatic power of flour as determined by the Lintner method is not the only factor that conditions diastasis in dough, is it not still possible that the respective effects of the papain and potassium iodate on the loaf volume in these baking tests were due to their effect on the liberation or depression of the flour amylase, largely beta amylase? Indeed, Stamberg and Bailey (*20*) found that added beta amylase produced poorer loaves of bread than the controls. Kosmin (*15*) also found that excessive starch hydrolysis is injurious to the baking qualities of flour but attributed this effect to the alpha amylase. This is at variance with the results of Stamberg and Bailey, who found that the baking qualities of bread were improved by the addition of alpha amylase (*20*). Kosmin based her conclusions on results obtained with sprouted-wheat flour, while Stamberg and Bailey used separate alpha and beta amylase preparations.

TABLE 2.—*Effect of various concentrations of papain on the development of diastatic power in flour*

Concentration of papain (percent)[1]	Diastatic power, in degrees Lintner, after—				
	3 hours	24 hours	48 hours	72 hours	144 hours
None	26.5	25.6			
.5	120.5	120.5			
.01	111.1	117.7			
.005	89.3	117.7	119.0		
.0025	66.7	85.5	109.0	115.0	120.5
.001	54.3	86.2	89.3	104.2	116.2
.0005	43.5	55.6	59.2	62.2	72.0

[1] On the basis of water added.

TABLE 3.—*Effect of various concentrations of potassium iodate on the development of diastatic power in flour*

Concentration of potassium iodate [1]		Diastatic power, in degrees Lintner, after—				
Molar	Percentage	3 hours	24 hours	48 hours	72 hours	144 hours
None	None	23.2	20.8	19.2	19.2	
.005	.11	11.3	3.0	3.0		
.00008	.0017	23.1	17.6	13.5	11.3	9.8
.00004	.00085	22.7	18.9	16.3	15.1	12.1
.00002	.00042	23.2	20.0	19.5	17.1	11.3

[1] On the basis of the water added.

The beneficial effect of natural aging of flour on the baking qualities of bread, which Balls and Hale (*2*, *3*) attribute to its inhibitive effect on the flour proteinases, may similarly be explained by its effect on the diastatic power of the flour, for the results presented here (controls in tables 2, 3, and 4) indicate that the diastatic power of flour gradually declines with time. The changes involved are relatively small, but they have been consistently verified in many tests.

In the concentration commonly used in bread making, however, sodium chloride also has a strong stimulating effect on the diastatic activity of flour (table 4). Even one-twentieth of the salt concentration used in the baking test causes a distinct stimulation of the diastatic power of flour. The maximum stimulation caused by salt, however, does not reach that caused by papain, and there are other differences between the action of papain and that of salt, which will be discussed elsewhere. The 2 percent of sodium chloride used in these baking tests did not cause any depression in loaf volume, notwithstanding the fact that it stimulated the diastatic activity of the flour. This would seem to exclude the possibility of explaining the results of the baking tests reported here by the effects of papain and potassium iodate on the stimulation or inhibition of the diastatic power of flour.

TABLE 4.—*Effect of various concentrations of sodium chloride on the development of diastatic power in flour*

Concentration of sodium chloride (percent)[1]	Diastatic power, in degrees Lintner, after—			Concentration of sodium chloride (percent)[1]	Diastatic power, in degrees Lintner, after—		
	3 hours	24 hours	48 hours		3 hours	24 hours	48 hours
None	33.3	29.4	26.7	.5	96.2	94.0	93.5
2.0	99.0	104.2	103.1	.25	86.2	88.5	87.7
1.0	96.2	100.0	101.1	.1	70.4	70.4	70.9

[1] On the basis of the water added.

SUMMARY

The results of baking tests show that there is a consistent interaction between various concentrations of papain and potassium iodate in their effect on the loaf volume of bread.

When added singly, some concentrations of potassium iodate and papain depress the loaf volume of bread, the extent of depression being directly related to the concentration. When added together, certain of these concentrations counteract each other.

While papain stimulates diastatic activity and potassium iodate depresses it, the action of these substances on the loaf volume of bread cannot be ascribed to their effect on the diastatic power of the flour since sodium chloride, which is always used in bread baking, also stimulates diastatic activity, yet causes no depression in loaf volume.

The results seem to support the theory of the mechanism of action of bread improvers advanced by Jørgensen and Balls and Hale.

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INHERITANCE OF FACTORS INFLUENCING SUCROSE PERCENTAGE IN BETA VULGARIS [1]

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INTRODUCTION

A more comprehensive knowledge of the manner in which factors affecting sucrose percentage are inherited in sugar beets (*Beta vulgaris* L.) should be of considerable aid to sugar-beet breeders. Although use has been made of the sucrose percentage of individual plants in selection work, there is little in the literature that throws light upon the nature of inheritance of the ability of sugar beets to produce sucrose. Accordingly the investigation reported in this paper was made with the object of determining how sucrose percentage is affected by genetic factors in crosses between inbred lines of sugar beets differing in respect to their mean sucrose percentage.

REVIEW OF LITERATURE

Bougy (*1*) [3] stated that in crosses of sugar beets with mangel-wurzels the sucrose percentage of the F_1 was higher when mangel-wurzels were used as the female parent than when sugar beets were so used. However, the results were found to depend to some extent upon the variety of mangel-wurzel used as a parent in the cross. His conclusion was based on limited data. In the segregating generations Bougy found that the plants were intermediate between the parents and that ordinarily they were closer to the parent higher in sucrose percentage. Certain roots surpassed the higher parent, but only very rarely was the sugar content of the lower parent recovered.

In later investigations, the F_1 progenies of reciprocal crosses between the Kuhn sugar beet × mangel-wurzel and mangel-wurzel × Kuhn sugar beet reported by Colin and Bougy (*2*) averaged, respectively, 11.2 and 7.6 percent of sucrose. These results were based on the average of 20 plants of the first cross and 18 plants of the second cross. At another location, Kuhn × mangel-wurzel and mangel-wurzel ×

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[3] Italic numbers in parentheses refer to Literature Cited, p. 171.

Kuhn F_1 crosses averaged 12.60 and 12.58 percent sucrose, respectively, in 1936, and 10.76 and 10.96 percent, respectively, in 1937. In 1937, at the same location 5 plants of the cross Vilmorin A sugar beet × mangel-wurzel and three plants of mangel-wurzel × Vilmorin A averaged 14.05 and 12.00 percent, respectively.

In the same paper Colin and Bougy reported four tests made in 1928 to 1931 in which crosses of sugar beets × mangel-wurzels and their reciprocals averaged 15.99 and 16.56 percent sucrose, respectively. The number of F_1 plants in each cross was not reported. The arithmetic average of the parental lines in these four tests was 12.27 percent, which was much below the average of the F_1 plants.

The same authors reported that in a total population of 76 F_2 plants no root was as high in sugar percentage as the highest of the sugar-beet roots, but that 5 of the hybrids exceeded the poorest sugar-beet root. No F_2 roots were as low in sugar percentage as the lowest mangel-wurzel.

Savitsky (*6*), in a test of 62 Russian varieties of sugar beets, stated that the variability of sugar percentage was small compared to the variation of all other characters. The average sucrose percentage in the roots of the varieties tested ranged from 17.01 ± 0.09 to 14.47 ± 0.20.

Hybrids produced by the "method of alternate planting of stecklings of the varieties crossed" were studied by Savitsky (*7*). Crosses of high × low or low × high sugar varieties produced in general F_1 progenies with a sucrose percentage near the arithmetic mean of the two parent varieties. In crosses of this type, the sucrose percentage of the higher parent was rarely obtained in the F_1.

Savitsky (*8*) studied the species *Beta vulgaris* L. and found that the sugar beet was highest in sucrose percentage, followed in order by white mangold,[4] semisugar beet, table beet, and mangel-wurzel. He believed that as a result of long-continued selection the sugar beet differs from primitive forms, not only in high sucrose content but also in its ability to retain this high sucrose content under adverse environmental conditions. F_1 hybrids between white mangolds and sugar beets were found to be higher in sucrose percentage than the mean of the parents, and frequently approached the sucrose percentage of the sugar beet. Backcrosses of the F_1 hybrid with sugar beets produced progenies with a sucrose percentage nearly equal to that of the sugar-beet parent. The high sucrose percentage of the sugar beet × mangold hybrids persisted in F_2 and failed to segregate as expected, there being a preponderance of individual F_2 roots of high sucrose percentage.

From his observations, Savitsky concluded that the mangold possessed certain basic genes influencing sucrose percentage which closely resembled those of the sugar beet, and that the mangold lacked certain modifying factors necessary for high sucrose percentage which have been retained in the sugar beet through selection. The genetic constitution of the mangold was tested by means of crosses with mangel-wurzels compared with crosses of sugar beets with mangel-wurzels. It was found that the F_1 cross of mangold with mangel-wurzel produced nearly the same sucrose percentage as the F_1 cross of sugar beets with mangel-wurzels. The slight difference in the two crosses was attributed to the presence of modifying factors contained in the sugar beet.

[4] The term "mangold" is normally used synonymously with "mangel-wurzel." As explained to Dr. G. H. Coons (via interpreter), and from the context of the paper, Dr. Coons believes that Savitsky refers to chard or chardlike plants.

In conclusion, Savitsky stated that a knowledge of beet genetics made it possible to synthesize a beet variety of high sucrose percentage in a few generations with only a limited number of plants.

As a result of correlation studies, Pack (*4*) found that the sucrose percentage of beets was positively and significantly correlated with such root characters as increased density, dry substance, extension of the shoulder beyond the first leaf scar, hardness of tissue, length, purity, refractometer reading, and roughness of skin. Top characters found to be associated with high sucrose percentage were darkness of leaf color, flatness of crown, and petioles of small cross-section area.

MATERIALS AND METHODS

The best inbred lines obtainable were used as parents in this study. Special emphasis was placed on as long a record of inbreeding as possible, but it was found that lines inbred for a period of years were not numerous. Two of the lines finally selected had a record of 5 or 6 years of inbreeding, and one line had been inbred for a shorter period but had originated from material that appeared homozygous in agronomic test plots.

It is generally recognized that sucrose percentage in the beet is influenced by environment as well as by genetic factors. For this reason it has been difficult to determine whether or not a line was homozygous until progeny tests have been made by inbreeding a number of plants of the line and determining the variations within and between the selfed progenies.

Seed and roots were obtained from two inbred strains,[5] one of which, called line 1 in this paper, had a pedigree starting in 1915 as follows: *s-s-s-s-gr-s-gr-gr-gr;* the other, called line 2 in this paper, had the pedigree *s-s-s-s-s-s-s-gr-gr*. In the production of these strains, space isolation (*s*) was relied upon to obtain selfing. The groupings (*gr*) also were made at isolated locations by combining the progeny of several roots. A detailed discussion of these lines, under the designations strain 1 and strain 9a, has been given by Stewart, Lavis, and Coons (*9*).

Roots were obtained from one additional inbred line, 4276,[6] which was reported to be highly self-fertile but very low in sucrose percentage. The line had been inbred for two generations. Each of the three lines selected possessed distinct morphological characters and could be easily distinguished when grown in the field.

Bulk seed [7] of lines 1 and 2 was used, i. e., seed from isolated group increases, as well as seed of the F_1, F_2, and first backcross generations. One lot of seed of a commercial brand, which could be classed as of intermediate type with respect to sucrose percentage and root yield, was used as a check throughout these tests.

The inbred lines selected, particularly lines 1 and 2, were believed to be relatively pure. The crossing program was begun before the lines themselves could be thoroughly tested.

Six roots of each of the three lines were transplanted in the greenhouse during the winter of 1936–37. All three possible crosses between

[5] These two strains originated in the leaf spot resistance breeding project and were furnished by Dewey Stewart, of the Division of Sugar Plant Investigations.

[6] This line, 4276, originated from breeding stocks in the curly top resistance breeding project; the roots were supplied by F. V. Owen, of the Division of Sugar Plant Investigations.

[7] Supplied by Dewey Stewart.

these lines were made by means of controlled pollination. Sturdy branches with 8 to 12 well-placed flower clusters were selected for emasculation. It was observed that the stamens were more easily removed from the larger florets and that a higher percentage set seed than when smaller and more slowly growing florets were used. The tip of the branch with the youngest florets was removed, and leaflets along the branch were removed also. When there was little difference in the size of the lateral flowers as compared with the central flowers, the entire group was emasculated; otherwise, the lateral flowers were removed. Usually less mechanical injury resulted from emasculation than from removing the entire lateral flower, and for that reason many of the lateral flowers were emasculated and later pollinated.

Branches with emasculated flowers were kept covered with light paper bags to exclude foreign pollen. The stigma lobes were usually well opened 4 or 5 days after emasculation, and pollination at that time proved most effective.

For the production of selfed seed, unemasculated branches on each plant were covered with bags before the flowers had opened. The amount of selfed seed produced on these original plants was not large enough to permit of planting in the field in 1937.

Seed of each of the three crosses was planted in soil in paper tubes $2 \times 2 \times 7$ inches in greenhouse flats early in the spring. These plants were transplanted to the field at Waseca, Minn., on May 22, 1937. The paper was removed from the core of soil and the core then planted in a hole of suitable size. Thus, minimum injury resulted to the roots of the young plants, and a high percentage of the transplants survived. The normal growth of the taproot was not seriously affected by this method of transplanting, and a higher percentage of the seedlings was saved than would have been the case had the seed balls been planted directly in the field.

The selfed seed of each line was planted in pots in the greenhouse, and the plants transferred to the field. In the fall they were removed to the greenhouse and again self-pollinated to continue the inbred lines.

Two roots of good size and shape were selected from the F_1 population of each cross, and after a short period of storage at a temperature of 34° F. they were replanted in the greenhouse.

Backcrossed seed was obtained during the winter of 1937–38, by means of controlled pollination, by the method used in making the original crosses, as described above. All three of the F_1 crosses, 1×2, 1×4276, and 2×4276, were backcrossed to the selfed plants of each of the parental lines of the original cross.

In the fall of 1938 a random sample of roots was selected from each of the parental lines, F_2 populations, and backcross populations. In addition, the highest and lowest testing individuals of each population were selected for further study. Since further study of the parental lines to determine the variability within each inbred line seemed desirable, selfed progenies of individual plants from the random lot selected from each inbred line were used to determine the variability in sucrose percentage existing within the lines. The random lots selected from within the segregating populations were used to show how rapidly types with various sucrose percentages could be fixed. Those roots with sucrose percentages at the extremes of the frequency

distributions were of particular interest, since it was possible that in the F_2 population individual roots relatively homozygous for sucrose percentage might have occurred.

The roots thus selected were planted in 8-inch pots in the greenhouse and bagged for selfing as the first flowers opened. Out of a total of 350 roots planted, 300 plants set seed, although in some instances the amount was small and in others the germination was low. Selfed seed of the 300 families was planted in the field in 1939.

Because of the small amount of seed, it was impossible to plant at customary rates and follow by thinning to one plant in each place. Rather it seemed advisable to make the smaller lots of seed go as far as possible by planting single seed balls and thus obtaining the maximum number of plants.

In 1937 the spacing employed was 36 by 30 inches. From a study of the effect of missing plants upon the weight of roots adjacent to a skip in 18 by 18 inch planting in commercial fields, it was found that the very wide spacings used in 1937 were probably unnecessary, hence a 20 by 20 inch spacing was used in 1938 and 1939.

As previously mentioned, the F_1 plants were started in the greenhouse and transplanted in the field in 1937. Since larger quantities of seed were available for the 1938 and 1939 plantings, they were made directly in the field, dropping one seed ball, or more, if the quantity available permitted, in each hill spaced 20 by 20 inches. The stands were thinned, at the six-leaf stage, to a single plant in each place.

Since the original lots of F_1 seed produced by controlled pollination consisted of 25 to 100 seed balls each, each cross was planted in a single row of from 25 to 52 plants, without replication. Every alternate row in the test plot was planted to the check variety to serve as a measure of the variability of the soil in the test plot.

In 1938, three replications of single-row plots 125 feet in length were used. One row of the check variety was employed in each replication, and since the results showed that there was little variation from one replication to the next, the data for the individuals of each population were brought together from all three replications without correction for block effect.

In 1939 a much larger plot was used. The 300 families were planted in two replications of single-row plots 41⅔ feet in length. The check variety was planted in every sixth row. Ten-beet composite samples were taken from each check plot, and the sucrose percentage was determined. As in previous years, the performance of the check with respect to sucrose percentage in the beet was strikingly uniform over the entire test plot, and it was unnecessary to make any correction for sucrose percentage between the two replications.

Table 1 summarizes the various populations that were grown in the field in each of the 3 years.

The individual roots were tested for sucrose percentage by the Sachs-Le Docte cold-water digestion method.

Environmental conditions favored normal growth each year. The check variety yielded 9.3, 17.6, and 17.0 tons per acre, with a mean sucrose content of 13.62, 13.07, and 10.92 percent for the years 1937, 1938, and 1939, respectively. Yield per acre was lower in 1937 than in 1938 or 1939, due in part to the wider spacing used that year. Sucrose percentage was reduced somewhat in the check, inbred lines,

and crosses because of the wide spacing used. Sucrose percentage was generally low in all fields in southern Minnesota in 1938.

TABLE 1.— *Lines,*[1] *crosses, and backcrosses used in study of inheritance of sucrose-percentage factors, 1937–39*

1937[2] F_1	1938			1939		
	P_2	F_2	Backcrosses	P_2	F_2	Backcrosses selfed
1 × 2	1	1 × 2	(1 × 2) × 1	1	1 × 2	(1 × 2) × 1.
1 × 4276	2	1 × 4276	(1 × 2) × 2	2	1 × 4276	(1 × 2) × 2.
2 × 4276	4276	2 × 4276	(1 × 4276) × 1	4276	2 × 4276	(1 × 4276) × 1.
Check	Check		(1 × 4276) × 4276	Check		(1 × 4276) × 4276.
			(2 × 4276) × 2			(2 × 4276) × 2.
			(2 × 4276) × 4276			(2 × 4276) × 4276.

[1] The symbols P_1, P_2, and P_3 indicate the first, second, and third generations of inbreeding, respectively, after the original roots were received.

[2] A very limited amount of P_1 seed was produced in 1937, and the P_1 plants were grown in a special nursery.

EXPERIMENTAL RESULTS

VARIABILITY OF PARENTAL LINES

Before discussing the breeding behavior of the F_1, F_2, backcrosses, and F_3 lines, an analysis of the breeding behavior, after further selfing, of the three parental lines used in this study will be given. Lines 1 and 2 had a previous record of at least five generations of inbreeding, while line 4276 had been inbred for only 2 years. These lines were inbred for another 3 years, with paper bags used as a means of excluding foreign pollen.

Twenty roots of the parental lines grown in 1938 were selected from lines 1, 2, and 4276. These were selfed in the greenhouse during the winter of 1938–39, and progenies of all roots that produced a sufficient quantity of seed were grown in the field in 1939. The data obtained from these progenies are shown in table 2.

The P_3 families within line 1 varied widely in mean sucrose percentage and in uniformity. Culture 1 was highest in mean sucrose percentage and the most uniform, while culture 14 was lowest in sucrose and the most variable. The difference (3.53 ± 0.45) between the mean sucrose percentage of these two cultures was highly significant, and the difference between the standard deviations was found to be significant by using Fisher's (*3*) test of significance of the difference between two variances. The parents of these two progenies had been selected in 1938 as high and low, respectively, in sucrose percentage. The mean sucrose percentages and standard deviations of the other cultures were distributed between the extreme limits set by cultures 1 and 14. Cultures 1 and 9 appeared to be relatively homozygous, since most of the sucrose percentages of the individual roots fell into two classes in each case. Cultures 2, 4, 8, 12, and 14 seemed relatively heterozygous. It appeared, therefore, that line 1 was not as homozygous as might have been expected from its history of inbreeding prior to the time it was obtained for use in this study.

The P_3 progenies were much more uniform in line 2 than in line 1. The difference in mean sucrose percentage between culture 17 and culture 22, the highest and lowest testing cultures, was 2.09 ± 0.27 percent.

The parent roots of these two progenies were higher and lower, respectively, in sucrose percentage, than the average. All the other cultures had similar mean sucrose percentages. The standard deviations of the different P_3 progenies of line 2 were relatively similar. These facts indicated that, while line 2 was not completely homozygous, it was much more uniform than line 1.

TABLE 2.—*Frequency distributions* [1] *and means of sucrose percentages of P_3 progenies of roots selected from parental lines*

Line and 1939 culture No.	Sucrose percentage of P_2 parent	Roots in sucrose-percentage class—											Total roots	Mean sucrose percentage of P_3 progeny	Standard deviation
		4	5	6	7	8	9	10	11	12	13	14			
Line 1:		*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*		
1	14.1						2	9	7	1			19	10.38±0.14	0.63±0.10
2	13.7	1			2	3	9	2	1		1		19	8.73± .41	1.80± .29
3	13.3				1	9	7	6	1	1			25	8.96± .23	1.15± .16
4	13.2			1	2	2	5	7				1	18	9.03± .41	1.74± .29
5	13.2			1		1	7	12	3				24	9.58± .23	1.13± .16
6	13.0						3	7	5	2			17	10.29± .21	.87± .15
7	12.5				1		5	7	3				16	9.58± .24	.94± .17
8	12.5		1	3		2	4	7					17	8.52± .42	1.71± .29
9	12.4					1	5	6	1				13	9.43± .20	.73± .14
10	12.1				1	3	8	3					15	8.83± .21	.79± .14
11	11.4				1	1	6	5	2				15	9.40± .27	1.03± .19
12	11.4	1		2	1	5	11	6					26	8.45± .29	1.47± .20
13	10.8					6	9	3		1			19	8.94± .22	.95± .15
14	10.7		4	6	6	1	2					1	20	6.85± .43	1.93± .31
Line 2:															
15	15.2							2	4	1			7	10.70± .29	.76± .20
16	14.8							6	13	4	1		24	10.93± .14	.71± .10
17	14.8							2	5	4	1		12	11.37± .21	.72± .15
18	14.2						1	11	6				18	10.28± .11	.46± .08
19	13.9							2	16	2			20	10.90± .09	.40± .06
20	13.8							7	6				13	10.39± .09	.33± .06
21	13.8							3	15	5			23	10.96± .13	.62± .09
22	9.8					3	7	7	1				18	9.28± .17	.73± .12
23	6.9							2	10	1			13	10.89± .17	.60± .12
Line 4276:															
24	11.2		2	3	4								9	6.20± .20	.61± .14
25	10.7	3	2										5	4.80± .19	.42± .13
26	10.1				2								2	6.70± .00	.00± .00
27	9.0		3	2									5	5.46± .12	.27± .09
28	8.9			2									2	5.70± .00	.00± .00
29	8.2				1	6	3	8	1				19	8.91± .24	1.03± .17
30	7.5	1	1			1							3	5.77± .93	1.61± .66
31	7.0			2									2	5.95± .26	.36± .18

[1] The frequency distributions in this and following tables were originally divided into 0.5-percent sucrose classes, from which means and standard deviations were calculated.

The number of plants in the P_3 progenies of line 4276 was small, with the exception of culture 29. Considerable difficulty was experienced in maintaining line 4276 from inbred seed produced under bags in the greenhouse, although the line was originally reported to be highly self-fertile. That culture 29 was more self-fertile and higher in mean sucrose percentage than the other cultures of line 4276 may have been the result of accidental cross-pollination in the previous generation, but it appeared similar morphologically to other cultures of the same line and its peculiar behavior was not suspected until the sucrose analyses were made. With the exception of culture 29, line 4276 appeared to be relatively homozygous for a low percentage of sucrose. The lack of correlation between the sucrose percentage of the P_2 parents and the mean sucrose percentages of their P_3 progenies ($r = -0.22$) indicated also that this line was relatively homozygous.

Line 4276 appeared about as uniform as line 2, if culture 29 is disregarded. This lack of uniformity of the parental lines made it necessary to interpret carefully the data obtained in F_1 and segregating generations of crosses 1×2 and 1×4276. The data obtained from cross 2×4276 have been considered the most reliable.

Correlations between the sucrose percentage of the P_2 mother beets and the mean of their P_3 progenies were calculated and found to be 0.59, 0.35, and −0.22 for lines 1, 2, and 4276, respectively. Only the first of these correlation coefficients was statistically significant, since the values of r required for odds of 19 : 1 were 0.53, 0.67, and 0.71, respectively. Although the number of comparisons for each line was small and the value of r required for significance was high in each case, the fact that there was a smaller association between the sucrose percentage of the P_2 mother beets and the mean of their P_3 progenies in lines 2 and 4276 than in line 1 furnished additional evidence that lines 2 and 4276 were more uniform than line 1.

TEST OF BULK SEED LOTS

Bulk seed of the parental lines 1 and 2, reciprocal F_1 crosses, the F_2 generation, and two backcrosses, together with the check variety, was planted in 1937 in single-row plots 132 feet long.[8] The data obtained from this test are given in table 3.

TABLE 3.—*Frequency distributions and means of sucrose percentages of parental lines, F_1 and F_2 crosses, and backcrosses grown from bulk seed in 1937*

Line or cross	Roots in sucrose-percentage class—										Total roots	Mean sucrose percentage	Standard deviation
	8	9	10	11	12	13	14	15	16	17			
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*		
1					2	26	8	4			40	13.28±0.11	0.68±0.08
2				8	15	5	5		1		34	12.30± .20	1.15± .14
1×2, F_1					13	14	4				31	12.55± .14	.79± .10
2×1, F_1	1			1	8	21	9				40	12.75± .17	1.08± .12
1×2, F_2			3	3	11	14	8	2			41	12.65± .19	1.22± .13
1×(1×2)				1	11	16	9	4			41	13.09± .15	.97± .11
(1×2)×1				3	12	16	7	3			41	12.71± .16	1.01± .11
Check			1	1	9	9	12	11	1	2	46	13.62± .21	1.42± .15

The difference in mean sucrose percentage of the two parental lines was not as large as expected, since Stewart et al. (*9*) had reported a difference of 2.95 percent in 1932. Parental line 1 was significantly more uniform than the check variety, while line 2 was not significantly less variable than the check. The difference between the standard deviations of the reciprocal F_1 crosses approached significance. Both F_1 crosses were numerically less variable than the check, but the difference in variability between the F_1 cross and the check was significant only when line 1 was employed as the female parent. The F_2 cross was not significantly less variable than the check, while the two backcrosses were similar in variability, both being significantly less variable than the check variety. The relative uniformity of lines 1 and 2, as grown from bulk seed, did not agree with that obtained on further selfing and reported in table 2.

[8] These lots of seed, except the check, had been supplied by Dewey Stewart from bulk lots of the parental lines and crosses.

The parental lines differed significantly in mean sucrose percentage, but did not agree with the classification obtained by further selfing as shown in table 2. Line 1, grown from bulk seed in 1937, was 0.98 ± 0.23 higher in mean sucrose percentage than line 2. When grown in 1938 from seed produced by two further generations of inbreeding, line 2 was 1.28 ± 0.09 higher in mean sucrose percentage than line 1 (see table 5). Presumably the genotype of the plants used as parents in this cross was not the same as the predominant type isolated by further inbreeding.

RECIPROCAL CROSSES

Reciprocal crosses between identical plants were made in order to check previous reports that reciprocal F_1 crosses may differ in sucrose percentage. These reciprocal crosses were planted in such a way that the results from paired F_1 reciprocals could be studied in a critical manner. The results are given in table 4.

TABLE 4.—*Sucrose percentage of reciprocal F_1 crosses between identical plants*

Cross	Roots	Sucrose	Difference
	Number	*Percent*	
1×2	15	13.62	
2×1	15	13.57	0.05

The difference between the mean sucrose percentage of the paired F_1 progenies of reciprocal crosses was not of sufficient magnitude to be statistically significant. Although the number of paired comparisons was small, it seems evident that when identical plants were crossed, the sucrose content of the progeny was not influenced by the direction in which the cross was made.

Since it seemed clear from this test that there was no difference between reciprocal crosses, no importance has been attached in this study to the direction in which the crosses were made. In this paper, a cross reported as 1×2, for example, may contain the reciprocal cross, 2×1, as well.

F_1 AND F_2 CROSSES MADE BY CONTROLLED POLLINATION UNDER BAGS IN THE GREENHOUSE

The three F_1 crosses produced by controlled pollination under bags in the greenhouse were grown in 1937 in single-row plots. The plants were spaced 36 by 30 inches, and no correction was made for missing plants, since the wide spacing reduced competition to such an extent that sucrose percentage would be affected but little. In 1938 sufficient seed of the parental lines was available for planting, and the parental lines were grown together with the F_2 crosses in single-row plots, replicated three times. The beets were spaced 20×20 inches in 1938, and skips in the stand were disregarded. The frequency distributions, means, and standard deviations for the parental lines, F_1 and F_2 crosses, and the check variety are given in table 5.

TABLE 5.—*Frequency distributions and means of sucrose percentages of parental lines and F_1 and F_2 crosses from seed produced by controlled pollination*

Line or cross	Year grown	Roots in sucrose-percentage class—												Total roots	Mean sucrose percentage	Standard deviation
		7	8	9	10	11	12	13	14	15	16	17	18			
		No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*		
1×2, F_1	1937						2	25	13	7				47	13.48±0.11	0.76±0.08
1×4276, F_1	1937						2	12	5					19	13.09± .16	.69± .11
2×4276, F_1	1937				8	9	10	5						32	11.32± .18	1.02± .13
Check	1937				1	1	9	9	12	11	1	2		46	13.62± .21	1.42± .15
1	1938					20	73	52	4					149	12.24± .06	.71± .04
2	1938				1	2	6	47	70	11				137	13.52± .07	.79± .05
4276	1938	12	62	56	11	3	1							145	8.51± .07	.86± .05
1×2, F_2	1938				8	52	103	124	53	8				348	12.49± .06	1.03± .04
1×4276, F_2	1938		3	7	17	43	65	30	7	2				174	11.59± .10	1.33± .07
2×4276, F_2	1938			5	40	51	27	11	1					135	10.93± .09	1.05± .06
Check	1938				2	6	38	59	47	14	3			169	13.07± .08	1.07± .06

Since it was impossible to produce sufficient seed by controlled pollination to permit growing the parental lines and F_1 and F_2 crosses the same year comparison had to be made of populations grown in 2 successive years. The same lot of check seed was used in both years and served as a measure of the relative sucrose percentage and variability in the 2 years.

The three parental lines were less variable in sucrose percentage than the check variety, and the variability was not significantly different. On further selfing, line 1 proved to be more variable than lines 2 and 4276, as shown previously.

The average sucrose percentage of the F_1 crosses grown in 1937 was 12.63. The average of the parents grown in 1938 was 11.42 percent, which was lower than the average of the F_1 crosses by 1.21 percent. The check, grown in both years, was lower in sucrose percentage in 1938 by 0.55 percent. It appears, therefore, that the F_1 crosses were in general slightly higher in sucrose percentage than the average of the parents. The average of the F_2 crosses was 11.67 percent sucrose, which is only slightly higher than the average of 11.42 for the parents grown in the same year.

The F_1 generations of crosses 1×2, and 1×4276 were no more variable than their parental lines, while the F_1 of cross 2×4276 was more variable than either parent. The F_2 populations of crosses 1×2, 1×4276, and 2×4276 were more variable than the F_1.

The variability, as expressed by the standard deviation of the F_2 populations, was highest in cross 1×4276. In cross 2×4276 the standard deviation of the F_2 population was relatively low, in spite of the fact that the mean difference in sucrose percentage between the parent lines was 5.01 percent and the maximum amount of segregation would be expected to occur in F_2. In this cross the segregation in F_2 reached neither the upper limit of the parent higher in sucrose percentage nor the lower limit of the parent lower in sucrose percentage.

CROSS 1 × 2

The frequency distributions and mean sucrose percentages of the parental lines, F_1, F_2, backcrosses, F_3 progenies of selfed F_2 plants, and selfed progenies of the backcrosses obtained from the cross of lines 1 and 2 are shown in table 6.

TABLE 6.—*Frequency distributions, means, and standard deviations of sucrose percentages of parental lines 1 and 2 and of F_1, F_2, and F_3 crosses and backcrosses of 1 × 2*

PLANTS GROWN IN 1937 AND 1938

Line or cross	Year grown	Roots in sucrose-percentage class—														Total roots	Mean sucrose percentage	Standard deviation
		4	5	6	7	8	9	10	11	12	13	14	15	16	17			
		No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*		
1×2, F_1	1937									2	25	13	7			47	13.48±0.11	0.76±0.08
Check	1937							1	1	9	9	12	11	1	2	46	13.62± .21	1.42± .15
1	1938								20	73	52	4				149	12.24± .06	.71± .04
(1×2)×1	1938								1	4	5	8	3			21	13.30± .23	1.04± .16
1×2, F_2	1938							8	52	103	124	53	8			348	12.49± .06	1.03± .04
(1×2)×2	1938							2	1	13	21	8				45	12.71± .13	.85± .09
2	1938							1	2	6	47	70	11			137	13.52± .07	.79± .05
Check	1938							2	6	38	59	47	14	3		169	13.07± .08	1.07± .06

F_3 PROGENIES OF SELFED F_2 PLANTS, GROWN IN 1939

1939 culture No.	Sucrose percentage of parent plant	Roots in sucrose-percentage class—														Total roots	Mean sucrose percentage	Standard deviation
		4	5	6	7	8	9	10	11	12	13	14	15	16	17			
		No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*		
32	14.0					1	1	9	11	2						24	11.43±0.19	0.91±0.13
33	13.9					1	2	9	4		1					17	11.14± .25	1.03± .18
34	13.8			1		4	4	3	3							15	9.97± .35	1.36± .25
35	13.8				1	1	4	1	1							8	9.76± .43	1.21± .30
36	13.5						3		3	1						7	11.13± .41	1.10± .29
37	13.3	1	3	4	5	3	1	2								19	7.70± .38	1.66± .27
38	13.3			1	4	6	6									17	9.94± .19	.79± .14
39	13.1					6	2									8	10.32± .21	.58± .14
40	13.1				1	4	3									8	10.14± .24	.68± .17
41	13.0			1	3	6	3	1								14	9.88± .30	1.12± .21
42	12.9						5	1	2							8	10.58± .26	.74± .18
43	12.8				1	3	14	8	1							27	10.22± .14	.74± .10
44	12.8					1	3	4	4	3			1			16	11.64± .38	1.54± .27
45	12.7				2	2	9	11		1						25	10.34± .21	1.04± .15
46	12.6					4	9	6								19	10.12± .17	.73± .12
47	12.5			1		2	3	4								10	8.85± .41	1.29± .29
48	12.5					1	3	3	7	4	1					19	10.62± .27	1.18± .19
49	12.4						1	1	6	3	4					15	11.40± .31	1.19± .22
50	12.2				1	2	4	5	6							18	9.59± .25	1.06± .18
51	12.1						4	7	4	3						18	10.32± .25	1.08± .18
52	12.0				2	2	7	3		1	1					16	9.17± .40	1.60± .28
53	12.0						2	11	17	5						35	10.70± .13	.79± .09
54	12.0				1		3		11	5	1					21	10.75± .30	1.36± .21
55	11.8						1	1	7	6	1	1				17	11.38± .26	1.09± .19
56	11.8		1		1	5	8	7	5	1						28	9.20± .27	1.42± .19
57	11.7				1	2	3	5	9							20	10.00± .27	1.23± .19
58	11.7					1	10	7	2							20	9.45± .15	.66± .10
59	11.6				1	1	3	7	9	2						23	10.09± .25	1.22± .18
60	11.4					3	2	4								9	9.09± .23	.70± .16
61	10.7						2	17	7							26	10.16± .10	.53± .07

SELFED PROGENIES OF BACKCROSS (1×2)×1

109	14.5					1		3	6	3						13	10.66±0.27	0.97±0.19
110	14.1							8	9	6						23	10.85± .16	.75± .11
111	13.0								1	9						10	11.85± .11	.34± .08
112	12.9					1	5	13	6							25	10.02± .15	.76± .11
113	12.5						3	6	8	1						18	10.39± .21	.87± .14
114	12.4					2	6	8	1							17	9.35± .19	.77± .13
115	12.3					1	5	22	8							36	9.91± .11	.67± .08
116	11.2						2	6	1							9	9.81± .23	.65± .15

TABLE 6.—*Frequency distributions, means, and standard deviations of sucrose percentages of parental lines 1 and 2 and of F_1, F_2, and F_3 crosses and backcrosses of 1 × 2*—Continued.

SELFED PROGENIES OF BACKCROSS (1×2)×2

1939 culture No.	Sucrose percentage of parent plant	Roots in sucrose-percentage class—														Total roots	Mean sucrose percentage	Standard deviation
		4	5	6	7	8	9	10	11	12	13	14	15	16	17			
		No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*		
117	14.1				1	1		8	7	10						27	10.79±0.20	1.02±0.14
118	14.1						1	3	8	9	3					24	11.30± .20	.97± .14
119	13.7							1	6	1	1					9	11.03± .25	.75± .18
120	13.6							7	12	2	1					22	10.79± .21	.97± .15
121	13.5							1	13	6						20	11.25± .13	.56± .09
122	13.3							10	7	10	2					29	11.06± .18	.99± .13
123	13.1						1	4	9	8	1					23	11.16± .19	.92± .14
124	13.1						2	11	10							23	10.29± .12	.60± .09
125	12.8					1	1	4	5	2						13	10.43± .26	.95± .19
126	12.8				1	6	14	6	2							29	8.96± .16	.88± .12
127	12.8							2	10	12	4					28	11.68± .18	.98± .13
128	12.6					1	3	10	5							19	10.02± .17	.75± .12
129	12.4					2	2	3	1							8	9.26± .31	.86± .22
130	12.1					2	4	11	6	1						24	9.93± .19	.94± .14
131	11.2						1	3	3							7	10.06± .32	.85± .23

The mean sucrose percentages of the parents, the F_1, and the F_2 generation were 12.88 ± 0.05, 12.93 ± 0.11, and 12.49 ± 0.06, respectively, the F_1 being reduced by 0.55 percent since the check was that much higher in 1937 than in 1938. The differences between the means of the parents, the F_1, and the F_2 in percentage sucrose was not great.

When the F_1 was backcrossed to line 1, the sucrose percentage was nearly equal to that of line 2, and the reciprocal backcross, $(1 \times 2) \times 2$, was more nearly like line 1 in sucrose percentage. Thus the backcrosses were not in accord with expectation, and the results could not be explained on the basis of the performance of the parental lines. Apparently the genotype of the plants used in these crosses was not truly described by the mean performance of the parent lines. The fact that line 1 was relatively heterozygous has been pointed out previously.

The parental lines and the F_1 appeared to be equally variable in their frequency distributions, while the F_2 was more variable than either the parents or the F_1. The backcross $(1 \times 2) \times 1$ was as variable as the F_2, while the difference between the standard deviations of the F_2 and the backcross $(1 \times 2) \times 2$ approached significance.

Of the 30 F_3 families of this cross, grown in 1939, 10 appeared to be as uniform in sucrose percentage as the more uniform P_3 lines of the parents, and 20 appeared to be less uniform. The mean sucrose percentages of each group were divided equally above and below the mean of the 30 families.

After allowance was made for the variability that existed within the parental lines, it seemed evident that certain families were as homozygous as the parental lines, while others were fully as variable as the F_2 population.

The mean sucrose percentages of lines 1 and 2 grown in 1939 were 9.07 and 10.63, respectively (table 2). Eight F_3 families had a mean

sucrose percentage equal to or greater than the higher sucrose parent. One of these, culture 53, was no more variable than the more uniform selfed progenies of line 1.

Progenies produced from selfed backcross plants were more uniform than the F_2, and in many cases appeared as uniform as the original parents, which would indicate that a single generation of backcrossing had tended definitely to reduce the genetic variability of the progeny.

CROSS 1 × 4276

Table 7 shows the frequency distributions and means of sucrose percentages of parental lines, F_1, F_2, and F_3 crosses, backcrosses, and selfed backcross progenies obtained from the cross of lines 1 and 4276.

TABLE 7.—*Frequency distributions, means, and standard deviations of sucrose percentages of parental lines 1 and 4276 and of F_1, F_2, and F_3 crosses and backcrosses of 1 × 4276*

PLANTS GROWN IN 1937 AND 1938

Line or cross	Year grown	Roots in sucrose-percentage class— 4	5	6	7	8	9	10	11	12	13	14	15	16	17	Total roots	Mean sucrose percentage	Standard deviation
		No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*		
1 × 4276, F_1	1937									2	12	5				19	13.09±0.16	0.69±0.11
Check	1937							1	1	9	9	12	11	1	2	46	13.62±.21	1.42±.15
1	1938								20	73	52	4				149	12.24±.06	.71±.04
(1 × 4276) × 1	1938							2	5	26	13	7				53	12.31±.13	.92±.09
1 × 4276, F_2	1938					3	7	17	43	65	30	7	2			174	11.59±.10	1.33±.07
(1 × 4276) × 4276	1938					1	9	19	11	3						43	10.13±.09	.59±.06
4276	1938				12	62	56	11	3	1						145	8.51±.07	.86±.05
Check	1938							2	6	38	59	47	14	3		169	13.07±.08	1.07±.06

F_3 PROGENIES FROM SELFED F_2 PLANTS, GROWN IN 1939

1939 culture No.	Sucrose percentage of parent plant	Roots in sucrose-percentage class— 4	5	6	7	8	9	10	11	12	13	14	15	16	17	Total roots	Mean sucrose percentage	Standard deviation
		No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*		
62	13.2				1		12	7	2							22	9.31±0.17	0.79±0.12
63	12.7		1		1	1	1	3	7	1						15	9.80±.50	1.92±.35
64	12.7						2	5	9	2						18	10.64±.18	.76±.13
65	12.5			1	1	4	5	5	1							17	8.88±.30	1.25±.21
66	12.4				3	6	7	3	2							21	8.65±.26	1.18±.18
67	12.4			1		1	3	2	5							12	9.62±.43	1.49±.30
68	12.1		1	2	3	7	4	7		1						25	8.46±.31	1.56±.22
69	12.1		2	2	1	4	2	4	3							18	8.39±.51	2.18±.36
70	11.9					1	4	3	1							9	9.48±.29	.87±.21
71	11.9				1		4	7								12	9.33±.27	.93±.19
72	11.8					2	5	3	7	4	1					22	10.40±.28	1.32±.20
73	11.6					6	10	1	1							18	8.84±.17	.70±.12
74	11.4			1		4	4	1	2							12	8.66±.37	1.27±.26
75	11.3				1		2	10	8	2	1			1		25	10.60±.31	1.54±.22
76	11.3		4	3	3	6	3	2								21	7.30±.36	1.65±.25
77	11.0				1	4	5	6	7		1					24	9.76±.29	1.41±.20
78	10.8					1	2	8		2						13	9.85±.32	1.14±.22
79	10.7				1	1	4		1							7	8.84±.46	1.22±.33
80	10.7			2	3	3	4			1						13	8.05±.43	1.56±.31
81	10.6				2	5		1								8	7.82±.31	.88±.15
82	10.6		1	2	2	6	3	1								15	7.73±.48	1.85±.34
83	10.6					5	6	6								17	8.96±.21	.89±.15
84	10.5			1	3	5	6	3	2							20	8.62±.28	1.25±.20
85	10.5	2	1			2	5	2	1							13	7.97±.64	2.30±.45

TABLE 7.—*Frequency distributions, means, and standard deviations of sucrose percentages of parental lines 1 and 4276 and of F_1, F_2, and F_3 crosses and backcrosses of 1 × 4276*—Continued.

SELFED PROGENIES OF BACKCROSS (1 × 4276) × 1

1939 culture No.	Sucrose percentage of parent plant	Roots in sucrose-percentage class— 4	5	6	7	8	9	10	11	12	13	14	15	16	17	Total roots	Mean sucrose percentage	Standard deviation
		No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*		
132	13.4				1		3	6	7	3						20	10.22±0.27	1.20±0.19
133	13.2			1	1	1	13	10	4	1						31	9.43± .20	1.12± .14
134	12.8					2	7	13	7							29	9.65± .17	.91± .12
135	12.5						2	4	6	2						14	10.52± .21	.80± .15
136	12.4				1	2	9	7								19	9.02± .18	.77± .12
137	12.4					9	8	3								20	8.62± .15	.65± .10
138	12.3						10	12	6							28	9.88± .15	.81± .11
139	12.1				2	4	9	3	1							19	8.78± .24	1.06± .17
140	12.0				2	2	2		1							7	8.41± .47	1.25± .33
141	11.8					6	8	8	3							25	9.32± .19	.97± .14
142	11.6		2	3	6	9	3	1	2							26	7.57± .33	1.69± .23
143	11.7				1	2	7	7	3		1					21	9.56± .27	1.23± .19
144	11.2				4	6	9	9	5		2					35	9.36± .24	1.44± .17

SELFED PROGENIES OF BACKCROSS (1 × 4276) × 4276

145	13.8						1	2	3	1	2					9	10.98±0.43	1.28±0.30
146	13.6					1	1	2	3	1						8	10.14± .49	1.37± .34
147	12.3					1	3	8	4	5						21	10.39± .23	1.07± .17
148	12.2				5	6	2	1								14	7.91± .23	.85± .16
149	11.2			2	1	4	1	1		2						11	8.52± .64	2.12± .45
150	11.0		2	2	2	2										8	6.26± .56	1.59± .40
151	10.5			4	1	4										9	6.98± .35	1.04± .25
152	10.4					5	7	9		1	1					23	9.50± .28	1.34± .20
153	10.3		5	4	4	2	2									17	6.49± .34	1.38± .24
154	9.9			1	1	5	10	1								18	8.51± .22	.94± .16
155	9.8		2	5	4	5	1									17	6.82± .29	1.20± .21
156	9.5			1	2	5										8	7.39± .33	.92± .23
157	9.4			1	2	5										8	7.39± .23	.65± .16
158	9.3			1	3	7	1	1								13	7.66± .29	1.03± .20
159	8.3		3	3	1			1		2						10	7.25± .91	2.87± .64

The mean sucrose percentage of the F_1 was 2.71 percent higher than the mean of the two parents. After allowance was made for the difference in sucrose percentage of the check for the 2 years, the net increase of the F_1 over the average of the two parents was 2.16 percent. The mean sucrose percentage of the parents and the mean of the F_2 could be compared directly since both progenies were grown the same year under the same conditions. The mean sucrose percentages of the parents and of the F_2 were 10.38 ± 0.05 and 11.59 ± 0.10, respectively. The difference (1.21 ± 0.11) was significant. The mean sucrose percentage of the F_1 backcrossed to the parent higher in sucrose percentage was equal to the sucrose percentage of the recurrent parent.

The F_1 cross was as uniform as the parental lines, and the F_2 was significantly more variable. The backcross (1 × 4276) × 4276 was less variable and the backcross (1 × 4276) × 1 slightly more variable than the recurrent parents, but both backcrosses were less variable than the F_2.

The range of values of the F_2 generation exceeded that of the higher parent by one class and failed by one class to reach the lower limit

of the lower parent. The progeny of the backcross to the higher parent was distributed in a manner similar to the higher parent, while the backcross to line 4276 did not recover the lowest percentage sucrose class of line 4276.

Twenty-four F_3 families were grown in 1939 from selfed seed of F_2 plants. Of these, 7 appeared to be as uniform as the parental lines, while 17 were more variable.

Some of the most uniform families, e. g., culture 64, were appreciably above the average in sucrose percentage, while others, e. g., culture 81, were considerably below the average. The same was true of the variable families; certain ones were high in mean sucrose percentage (culture 75) and others were low (culture 85).

Progenies produced from selfed backcross plants were in many instances as variable as the F_2 generation, while in other instances they were as uniform as the parents. As in the case of the cross 1×2, there was no apparent relationship between the mean sucrose percentage of the F_3 families and the variability within the families.

The mean sucrose percentage of the 13 progenies of $(1 \times 4276) \times 1$ was 9.26 percent. This was slightly higher than the mean of the 24 F_3 progenies. The mean of the 15 selfed progenies of $(1 \times 4276) \times 4276$ was only 8.15 percent. While the variation between the progenies was relatively large, backcrossing produced a pronounced shift in sucrose percentage, on the average, in the direction of the recurrent parent.

CROSS 2 × 4276

The frequency distributions and means of the sucrose percentages of the parental lines, F_1, F_2, and F_3, and backcrosses of the cross 2×4276 are shown in table 8. Since the two lines used in this cross were relatively homozygous, as demonstrated previously, it would be expected that this cross would provide more critical data than the crosses 1×2 and 1×4276.

TABLE 8.—*Frequency distributions and means of sucrose percentage of parental lines 2 and 4276 and F_1, F_2, and F_3 crosses and backcrosses of 2×4276*

PLANTS GROWN IN 1937 AND 1938

Line or cross	Year grown	Roots in sucrose-percentage class—														Total roots	Mean sucrose percentage	Standard deviation
		4	5	6	7	8	9	10	11	12	13	14	15	16	17			
		No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*		
2×4276, F_1	1937							8	9	10	5					32	11.32±0.18	1.02±0.13
Check	1937							1	1	9	9	12	11	1	2	46	13.62±.21	1.42±.15
2	1938							1	2	6	47	70	11			137	13.52±.07	.79±.05
(2×4276) ×2	1938						2	4	10	26	23	10	3			78	12.33±.14	1.23±.10
2×4276, F_2	1938						5	40	51	27	11	1				135	10.93±.09	1.05±.06
(2×4276) ×4276	1938					2	13	11	15	3						44	9.98±.15	1.01±.11
4276	1938				12	62	56	11	3	1						145	8.51±.07	.86±.05
Check	1938							2	6	38	59	47	14	3		169	13.07±.08	1.07±.06

TABLE 8.—*Frequency distributions and means of sucrose percentage of parental lines 2 and 4276 and F_1, F_2, and F_3 crosses and backcrosses of 2 × 4276*—Continued

F_3 PROGENIES OF SELFED F_2 PLANTS, GROWN IN 1939

1939 culture No.	Sucrose percentage of parent plant	Roots in sucrose-percentage class— 4	5	6	7	8	9	10	11	12	13	14	15	16	17	Total roots	Mean sucrose percentage	Standard deviation
		No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*		
86	13.2							5	11	10						26	11.10±0.15	0.76±0.11
87	13.1				1		3	5	4	2						15	10.03± .31	1.19± .22
88	12.6							2	2	3						7	11.20± .39	1.04± .28
89	12.4				1	1	4	10	5							21	9.77± .25	1.13± .17
90	12.3						3	4	2	2						11	10.29± .31	1.04± .22
91	12.2			3	4	3	1		1							12	7.49± .47	1.62± .33
92	12.0			1			7	2	3							13	9.28± .33	1.19± .23
93	11.7		1			2	3	7	3	1						17	10.05± .29	1.18± .20
94	11.6			2	3	3	1	1								10	7.60± .39	1.22± .27
95	11.6						5	10		1						16	9.73± .19	.76± .13
96	11.5		2	1	6	3	1									13	7.05± .33	1.18± .23
97	11.3		1	2	4	8	5	2								22	8.60± .26	1.21± .18
98	11.3				4	1	7	1								13	8.24± .29	1.05± .21
99	11.0				3	6	11	9	3	1	1					34	9.21± .22	1.31± .16
100	11.0				1	3	8									12	8.58± .19	.64± .13
101	11.0				1	3	3									7	8.13± .32	.84± .22
102	10.9				3	7	3									13	8.05± .21	.75± .15
103	10.5		1	1	1	5	2	1								11	7.88± .40	1.33± .28
104	10.5			1	3	6	3	4								17	8.29± .29	1.18± .20
105	10.4				7	5	3	2								17	7.99± .22	.90± .15
106	10.3		1	2	4	5	5	2								19	7.79± .35	1.53± .25
107	10.2			1	1	6	6	6	3	1						24	9.08± .29	1.43± .21
108	10.1		2		1	8	2									13	7.39± .36	1.32± .26

SELFED PROGENIES OF BACKCROSS (2×4276)×2

160	13.0				3	6	7	3								19	8.46±0.22	0.98±0.16
161	12.9				1	3	6	1								11	8.56± .21	.71± .15
162	12.9	3		2	1	10	2	2								20	7.22± .42	1.87± .30
163	12.3				4	2	6	8		1						21	8.99± .27	1.22± .27
164	12.2						3	7		1	1					12	10.12± .35	1.22± .25
165	12.1				2	8	2	2	1							15	8.47± .25	.98± .18
166	11.8			1	1	3	6	4								15	8.77± .32	1.24± .23
167	11.5			1	1	7	12	4	2		1					28	8.97± .25	1.30± .17
168	11.5			1		6	6	8	2							23	9.05± .25	1.18± .17
169	11.4				1	3	9	6								19	9.04± .18	.80± .13
170	11.4			2	3	8	3	7	4		1					28	8.90± .33	1.72± .23
171	11.4			1	2	4	1	4	2							14	8.81± .39	1.44± .27
172	10.8		1	2	5	1	1									10	6.65± .34	1.07± .24

SELFED PROGENIES OF BACKCROSS (2×4276)×4276

173	11.4	1	4	7	7	4	4									27	6.70±0.26	1.34±0.18
174	11.0			1	2	3	3	1								10	8.05± .39	1.25± .28
175	10.9					7	13	3	3							26	9.03± .19	.95± .13
176	10.9		2	5	3	1										11	6.25± .24	.79± .17
177	10.8	1				4	2		1							8	7.95± .71	2.00± .50
178	10.8			2	3	4	4	2								15	7.93± .30	1.18± .22
179	10.7				2	1	5	4	1							13	9.01± .32	1.16± .23
180	10.7		2	4	9	2	3									20	6.98± .26	1.15± .18
181	10.5			2	7	5	1									15	7.20± .19	.73± .13
182	10.4			3	11	9	2	1								26	7.51± .19	.96± .13
183	9.8		1	5	2	1										9	6.42± .24	.71± .17
184	9.5		1	5	5	2										13	6.51± .24	.85± .17
185	9.4	1	2	2	3	2										10	6.20± .44	1.39± .31
186	9.2	4	1	8	8	3	1	1								26	6.39± .30	1.52± .21
187	9.1	1		3	6	1										11	6.38± .36	1.19± .25
188	8.8		5	7	18	8										38	6.79± .16	.96± .11

The F_1 generation of the cross 2×4276 grown in 1937 tested 11.32± 0.18 percent sucrose compared with 11.02±0.5 percent as an average of the two parents grown in 1938. Since the check variety averaged 0.55 percent higher in 1937 than in 1938, it may be concluded

that the F_1 was not significantly different from the mean of the parents. The F_2 generation averaged 10.93 ± 0.09 percent sucrose as compared with an average of 11.02 ± 0.05 for the parental lines.

Each of the backcrosses might be expected to have a mean sucrose percentage equal to three-fourths of the sucrose percentage of the recurrent parent plus one-fourth of the sucrose percentage of the nonrecurrent parent. Each backcross closely approached the expected value, exceeding it only 0.06 and 0.22 percent in the backcrosses to the higher and lower testing parent, respectively.

The F_2 and both backcrosses were more variable than the parents. Six of the F_3 families were as uniform in sucrose percentage as the original parents, while many of the families were fully as variable as the F_2 generation. The variability among the progenies from the selfed backcross mothers was very similar to the variability found among the F_3 families. It appeared that lines no more variable than the parents can be isolated relatively easily in the F_3. Only 2 of the 23 F_3 progenies had a mean sucrose percentage as great as the high-sucrose parent. One of these, culture 86, also was no more variable than the parents. No F_3 lines were found in which the sucrose percentage was as low as in the low-sucrose parent, if culture 29 in line 4276 is disregarded (table 2).

When the F_1 was backcrossed to the high-sucrose parent, line 2, the mean of the selfed backcross progenies was 8.62 percent, a slightly lower value than the average (8.82 percent) obtained from the 23 F_3 families. Backcrossing to the low-sucrose parent resulted in a mean of 7.21 percent for the selfed backcross progenies, which was decidedly lower than the mean of the F_3. In cultures 176, 183, and 184, the mean sucross percentage was essentially the same as that of the low-sucrose parent and the variability was very little or no greater.

CORRELATION BETWEEN SUCROSE PERCENTAGE OF PARENT AND MEAN OF THE PROGENY

Correlation coefficients were calculated for sucrose percentages of roots of parent plants selected from segregating populations and the means of their progenies. Correlation coefficients are shown in table 9.

TABLE 9.—*Coefficients of correlation of sucrose percentages of roots of F_2 plants and means of F_3 progenies and between backcrosses and means of the selfed progenies*

Cross	Generations correlated	Comparisons	r [1]
		Number	
1 × 2	F_2 and F_3	30	0.1757
1 × 4276	do	24	.4425
2 × 4276	do	23	.6859**
(1 × 2) × 1	Backcrosses and progeny	8	.5141
(1 × 2) × 2	do	15	.5619*
(1 × 4276) × 1	do	13	.4672
(1 × 4276) × 4276	do	15	.7099**
(2 × 4276) × 2	do	13	.0459
(2 × 4276) × 4276	do	16	.5519*
Average			.4659**

[1] *Exceeds 5-percent level of significance; **exceeds 1-percent level of significance.

The correlation coefficients between the sucrose percentage of F_2 mother roots and the mean of the F_3 progenies were not significant in crosses 1×2 or 1×4276. In cross 2×4276 the correlation coefficient was highly significant. Sucrose percentages of the roots of the backcrossed plants were significantly correlated with the mean sucrose percentage of the progenies in three cases, and nonsignificantly correlated in three others. All correlation coefficients were positive.

Rider (*5*) described a method of using χ^2 for testing the homogeneity of a group of correlation coefficients. Applying this test for homogeneity, it was found that $\chi^2=9.51$ and $P=0.30$, indicating that the correlation coefficients were homogeneous and could be considered as arising from a single population. An average correlation coefficient of 0.4659 was obtained by transforming r to z as outlined by Fisher (*3*), which exceeded the value required for odds of 99 : 1. Selection of roots with a high percentage of sucrose, from segregating populations, would aid in the isolation of F_3 progenies high in sucrose percentage.

DISCUSSION

Data obtained from the three crosses studied indicated that the F_1 was approximately equal to the mean of the two parents in sucrose percentage. The only exception was in the case of cross 1×4276, in which the F_1 was higher than the mean of the parents, probably due to the fact that the genotype of the plants in line 1 used in the cross was not truly represented by the mean of the line. Line 1 was the most variable of the three lines used in these crosses. In the F_2, the mean sucrose percentage was slightly but not significantly higher than the mean of the parents. When the parents did not differ greatly in sucrose percentage, the extremes of both parental types were recovered in the distribution of the F_2 sucrose percentages.

F_3 families were obtained in which the mean sucrose percentage was similar to that of the high-sucrose parent, and several of these families were no more variable than the parental lines. In two cases, reciprocal backcrosses produced progenies with mean sucrose percentages which approached that of the recurrent parent. This was especially true of the cross between the two most uniform parental lines, 2 and 4276. From the selfed backcrosses progenies were obtained that were like the recurrent parent in mean sucrose percentage and uniformity.

Since F_3 families as uniform in sucrose percentage as the parent families were obtained frequently in all three crosses, it might appear that this quality was inherited in a relatively simple manner. On the other hand, these less variable families had mean sucrose percentages that in general fell within the range of the P_3 families of the parent higher in sucrose percentage. In no instance were found F_3 families of uniform sucrose percentage that fell in the range of P_3 familes of line 4276. Thus it appears that it may not be easy to recover in F_3 the sucrose type of the parents and that the actual number of genetic factors influencing sucrose percentage may be fairly great. It seems doubtful if there are genetic factors that influence only sucrose percentage; it is more probable that the physiologic processes of the plant are controlled genetically and that these processes in turn control the production and storage of sucrose. It also seems probable, as shown by Pack (*4*), that many factors which influence principally morpho-

logic characters of the plant may influence to a greater or less extent the sucrose percentage of the beet.

The ability of a sugar beet to store sucrose appears to depend upon the interaction of many genetic factors, some of which exert an important influence and some a relatively unimportant influence upon its expression. From the data obtained from these crosses, it seems that it should be possible to retain the desirable sucrose percentage of one inbred when crossed with other inbreds having a lower sucrose percentage but excelling in other characteristics.

SUMMARY

A study was made of the inheritance of sucrose factors in crosses between inbred lines of beets differing in percentage of sucrose. The average sucrose percentage of the F_1 and F_2 generations was approximately equal to the mean of the parents, being very close in the cross of the two most uniform lines. F_3 progenies and progenies of selfed backcrosses were obtained that were no more variable than the parental inbreds. Some of these progenies were as high in mean sucrose percentage as the high-sucrose parent, while others were significantly lower. When the parental lines differed widely in sucrose percentage, no F_3 progenies or progenies of selfed backcrosses were obtained that were as uniform as the parents and as low in mean sucrose percentage as the low-sucrose parent. It appeared that relatively homozygous lines of desirable sucrose percentage could be obtained comparatively easily. The ability to store sucrose was found to be inherited in a quantitative manner and to be dependent upon the interaction of several genetic factors.

The correlation between the sucrose percentage of mother beets selected from segregating populations and the means of their inbred progenies was found to be highly significant. Roots with a high percentage of sucrose, selected from segregating populations, were found to produce, as an average, F_3 progenies high in sucrose percentage.

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MATHEMATICAL MODEL OF EMBRYO ABORTION IN PHILLIPS CLING PEACHES[1]

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INTRODUCTION

Embryo abortion as indicated by a change in the color of the embryo and of the integuments of the ovule is closely associated with certain phenomena of development of peaches such as the gumming of the Phillips Cling.[2] Such evidence of embryo abortion is easy to observe but it may have taken place after the abortion itself had occurred. Thus it may be extremely difficult, if not impossible, to determine the time of abortion of the ovule and embryo by direct observation. It is nevertheless important to know the time and intensity of the tendency to abort both for reasons of practical control and theoretical connections with other related phenomena such as pit hardening and gumming.

It is possible to observe the frequency distributions of the lengths of normal embryos at intervals throughout the growing season and the frequency distribution of the lengths of the aborted embryos at harvest time. From these data it is possible to deduce the probability of abortion as a function of time. The probability function of abortion refers to the proportion of the total number of aborted embryos for the season that will occur in any interval of time. The probability of gumming can also be observed. Since abortion of embryos and gumming are so closely associated in Phillips Cling, we should find that the probability of gumming is much the same as the probability of abortion but may be shifted in point of time. Data are not now available for checking, except roughly, the agreement of the probability functions of abortion and gumming.

The purpose of this paper is to derive a mathematical model of embryo abortion on the basis of certain plausible assumptions. If the mathematical model agrees closely with the observed facts, then the assumptions may be believed to approximate the truth, and the model may be studied to shed further light on the occurrence of aborted embryos. For instance, from the mathematical model, we can compute when the susceptible period for abortion begins; when the probability of abortion is greatest; when any given percent of the abortions will occur; the relations of abortion to pit hardening, gumming, and split pit; and the like.

DEVELOPMENT OF MODEL

Let us assume that the lengths of normal embryos are distributed, at any time, according to a Pearson's type III curve, and that abortion does not cause a change in the measured lengths of the embryos.

[1] Received for publication April 23, 1941.

[2] DAVIS, LUTHER D. The GUMMING OF PHILLIPS CLING PEACHES. Hilgardia 11:1-34, illus. 1937.

Let

$p(t)$=probability of abortion at time, t
$m(t)$=mean length of normal embryos at time, t
$\sigma(t)$=standard deviation of the lengths of the normal embryos at time, t
$\alpha(t)$=third moment of the frequency distribution of the lengths of the normal embryos divided by the standard deviation cubed at time, t

Then the joint frequency distribution of the aborted embryos with respect to time, t, and length, s, is

$$\frac{p(t)}{\sigma(t)\sqrt{2\pi}}\, y_0(t) \left(1+\frac{\alpha(t)}{2\sigma(t)}(s-m(t))\right)^{\frac{4}{[\alpha(t)]^2-1}} e^{-\frac{2(s-m(t))}{\alpha(t)\sigma(t)}} \qquad (1)$$

on the basis of the stated assumptions. Time, t, is measured in weeks, and length, s, is measured from the beginning of the range. $y_0(t)$ is a function of t such that the integral of (1) from 0 to ∞ with respect to s is $p(t)$. $y_0(t)$ is approximately

$$\left(1-\frac{[\alpha(t)]^2}{48}\right)$$

Since the integral of $p(t)$ from the time of the first abortion to harvest-time with respect to t is 1 the total volume under the surface (1) is 1.

Let

t_0=time of abortion of the first embryos
t_∞=harvest time
$F_0(s)$=observed frequency distribution of the lengths of the aborted embryos at harvest time
$F(s)$=theoretical frequency distribution of the lengths of the aborted embryos at harvest time
${}_0M_i$=moments about the mean of $F_0(s)$
${}_0M'_i$=moments about the beginning of the range of $F_0(s)$
${}_tM_i$=moments about the mean of $F(s)$
${}_tM'_i$=moments about the beginning of range of $F(s)$

Then

$${}_tM_i' = \int_{t_0}^{t_\infty} p(t)\left[\int_{\frac{-2}{\alpha(t)}}^{\infty} \frac{1}{\sqrt{2\pi}}\, y_0(t) \quad (\sigma(t)s+m(t))^i \cdot\left(1+\frac{\alpha(t)}{2}s\right)^{\frac{4}{[\alpha(t)]^2}-1} e^{-\frac{2s}{\alpha(t)}}\, ds\right] dt \qquad (2)$$

If we assume that ${}_tM_i'={}_0M_i'$ then (2) serves to determine $p(t)$. Suppose that $p(t)$ is determined by its first four moments. Then we need

$${}_0M_1' = \int_{t_0}^{t_\infty} p(t)\, m(t)\, dt \qquad (3)$$

$${}_0M_2' = \int_{t_0}^{t_\infty} p(t)\,([\sigma(t)]^2+[m(t)]^2)\, dt \qquad (4)$$

$$ {}_0M_3' = \int_{t_0}^{t\infty} p(t)\left([\sigma(t)]^3\alpha(t) + 3[\sigma(t)]^2 m(t) + [m(t)]^3\right)dt \tag{5} $$

$$ {}_0M_4' = \int_{t_0}^{t\infty} p(t)\left\{[\sigma(t)]^4\left(3+3\frac{[\sigma(t)]^2}{2}\right) + 4[\sigma(t)]^3 m(t)\alpha(t) + 6[\sigma(t)]^2[m(t)]^2 + [m(t)]^4\right\}dt \tag{6} $$

Let

$$ M_1(t) = m(t) \tag{7} $$

$$ M_2(t) = [\sigma(t)]^2 + [m(t)]^2 \tag{8} $$

$$ M_3(t) = [\sigma(t)]^3\alpha(t) + 3[\sigma(t)]^2 m(t) + [m(t)]^3 \tag{9} $$

$$ M_4(t) = [\sigma(t)]^4\left(\frac{6+3[\alpha(t)]^2}{2}\right) + 4[\sigma(t)]^3 m(t)\alpha(t) + 6[\sigma(t)]^2[m(t)]^2 + [m(t)]^4 \tag{10} $$

and assume that

$$ M_i(t) = a_{i0} + a_{i1}t + a_{i2}t^2 + a_{i3}t^3 + a_{i4}t^4, \ (i=1, 2, 3, 4) \tag{11} $$

Let u_i' $(i=1, 2, 3, 4)$ be the moments of $p(t)$ about some convenient point, then the u_i' are determined from

$$ {}_0M_i' = a_{i0} + a_{i1}u_1' + a_{i2}u_2' + a_{i3}u_3' + a_{i4}u_4', \ (i=1, 2, 3, 4) \tag{12} $$

The moments of $p(t)$ about its mean, denoted by u_i $(i=1, 2, 3, 4)$, are then easily determined. In most cases $p(t)$ can then be satisfactorily represented by the corresponding Pearson curve.

APPLICATION TO OBSERVED DATA

The data on the constants of the frequency distributions of the normal embryos and the functions (7), (8), (9), and (10) for an orchard in Sutter County, Calif., for the season of 1939 are given in table 1. These data were supplied by Professor L. D. Davis and were collected as described [3] by him.

TABLE 1.—*Data for illustrating the mathematical model of peach abortion, Sutter County, Calif., 1939*

Date	Number observed	$m(t)$	$\sigma(t)$	$\alpha(t)$	$M_2(t)$	$M_3(t)$	$M_4(t)$
May 23	200	4.56	1.26	0.27	22.38	117.08	648.2
May 30	200	8.50	1.88	.00	75.78	704.25	6,789.7
June 6	150	12.24	2.04	−.86	153.98	1,979.28	25,899.9
June 13	200	15.22	1.49	−.52	233.87	3,625.34	56,658.7
June 20	200	15.32	1.37	−.46	236.58	3,680.72	57,669.1
June 30	200	16.23	1.04	−.17	264.49	4,327.66	71,087.0
July 7	200	16.14	1.06	.00	261.62	4,258.87	69,620.0

The detailed frequency distributions of the normal embryos at dates throughout the season are given in table 2. These distributions are skewed in one direction, then symmetrical, then skewed in the other

[3] DAVIS, L. D. SIZE OF ABORTED EMBRYOS IN THE PHILLIPS CLING PEACH. Proc. Amer. Soc. Hort. Sci. 37: 198–202, illus. 1939.

direction, and then symmetrical again. Pearson's type III curves are the simplest that can take account of these changes in form.

TABLE 2.—*Frequency distribution of normal embryos at specified dates, Sutter County, Calif., 1939*

Class intervals (millimeters)	Frequency distributions on—						
	May 23	May 30	June 6	June 13	June 20	June 30	July 7
0.0–0.9							
1.0–1.9	2						
2.0–2.9	14						
3.0–3.9	44	2					
4.0–4.9	65	6					
5.0–5.9	48	5					
6.0–6.9	21	27	4				
7.0–7.9	5	34	3				
8.0–8.9	1	42	5				
9.0–9.9		38	9				
10.0–10.9		29	9	2	1		
11.0–11.9		10	21	4	4		
12.0–12.9		5	34	9	5		
13.0–13.9		2	37	18	16	4	4
14.0–14.9			20	39	36	15	19
15.0–15.9			8	58	71	52	56
16.0–16.9				51	47	75	75
17.0–17.9				17	11	48	38
18.0–18.9				1	6	6	8
19.0–19.9				1			
Total	200	200	150	200	200	200	200

The observed frequency distribution of the lengths of aborted embryos at harvesttime is given in table 3. It should be noted that the centers of the class intervals in tables 2 and 3 are at the $x.45$ millimeter marks, where x stands for the integers 0–19.

The constants for $F_0(s)$ are

$$ {}_0M_1' = 10.71 \qquad {}_0M_2 = 8.36 \qquad {}_0M_3 = -22.42 $$
$$ {}_0M_4 = 246.89 \qquad {}_0\beta_1 = 0.86 \qquad {}_0\beta_2 = 3.53 $$

The $F_0(s)$ for Sutter County is unusually skewed, as was pointed out by Davis, and the corresponding probability distribution for gumming is not available. The $p(t)$ obtained for these data may be expected to differ somewhat from the probability distributions of gumming obtained for other years when $F_0(s)$ was found to be symmetrical.

In computing (7), (8), (9), and (10) the polynomials were made to take on the first three values and the average value of each of the last two pairs of values in table 1. This procedure was used because $p(t)$ weights the first values much heavier than the later values. Usual least-squares technique gives too much weight to the less important later intervals unless polynomials of higher degree are used. But higher degree polynomials have a tendency to change erratically in the important time interval. The origin for t is taken to be June 13. The reference date for these data is May 22.

The equations for determining the u'_t are

$$ 1.1628u_1' - .8076u_2' + .0382u_3' + .0375u_4' = -3.9518 $$
$$ 46.7243u_1' - 17.1908u_2' - .6923u_3' + 1.0173u_4' = -93.1255 $$
$$ 1{,}032.9630u_1' - 239.6403u_2' - 37.2680u_3' + 14.3985u_4' = -1{,}725.5516 $$
$$ 20{,}422.3878u_1' - 2{,}474.3933u_2' - 988.2735u_3' + 121.1640u_4' = -29{,}491.4488 $$

TABLE 3.—*Frequency distribution of the lengths of aborted embryos at harvesttime Sutter County, Calif., 1939*

Class interval (millimeters)	Number Observed	Class interval (millimeters)	Number observed
1.0–1.9	4	10.0–10.9	78
2.0–2.9	3	11.0–11.9	71
3.0–3.9	16	12.0–12.9	117
4.0–4.9	10	13.0–13.9	86
5.0–5.9	17	14.0–14.9	42
6.0–6.9	18	15.0–15.9	5
7.0–7.9	28	16.0–16.9	2
8.0–8.9	40		
9.0–9.9	59	Total	596

Whence

$$u_1' = -1.02 \qquad u_2' = 4.20$$
$$u_3' = 1.56 \qquad u_4' = 27.46$$

and

$$u_2 = 3.15 \qquad u_3 = 12.26 \qquad u_4 = 56.74$$
$$\beta_1 = 4.81 \qquad \beta_2 = 5.72 \qquad \kappa = -1.20$$

The constants of $p(t)$ call for a Pearson curve of type 1 which is U-shaped. This means that $p(t)$ is heavy on each end and light in the middle. A U-curve cannot be considered as a satisfactory representation of $p(t)$ since it is known that $p(t)$ becomes zero at the ends of its range. A probability distribution that has the required characteristics and approximately the desired moments was constructed by trial and error and is given in table 4. For comparison the probability distribution of gumming for the same orchard but for the season of 1936 is given. The main difference between the two distributions is the hump on the tail of the $p(t)$ for 1939. This hump causes the skewness of $F_0(s)$ for 1939.

Since $u_1' = -1.02$, the mean of $p(t)$ is 1 week before June 13. The end of the range is not definitely determined by the moments of $p(t)$, but from table 4 it appears that the start of abortion must coincide very closely with the beginning of pit hardening. The fact that 2 percent is given in the first class interval of $p(t)$ does not mean that abortion starts at the beginning of that week but that it starts sometime within the week. If the class marks of the probability distribution of gumming were shifted a day or so the first parts of the two distributions would be even more nearly alike.

TABLE 4.—*Comparison of p(t) for Sutter County, Calif., 1939, and the probability of gumming, Sutter County, 1936*

Period from beginning of range (weeks)	Percent of total aborted embryos [1]	Percent of total gummed fruit [2]	Period from beginning of range (weeks)	Percent of total aborted embryos [1]	Percent of total gummed fruit [2]
1	2.0		7	3.0	1
2	50.0	41	8	6.0	1
3	25.8	32	9	1.2	1
4	9.0	15			
5	2.0	7	Total	100.0	100
6	1.0	2			

[1] Aborting distribution: $u'_1 = 0.11$, $u_2 = 3.24$, $u_3 = 12.28$, $u_4 = 57.02$.
[2] Gumming distribution: $u_2 = 1.71$, $u_3 = 4.23$, $u_4 = 23.40$.

DISCUSSION

The usual situation is for the frequency distributions of the lengths of aborted embryos to be symmetrical and approximately normal. In such cases the probability distribution of abortion can be adequately represented by a Pearson curve of type 1_1 of limited range which approaches the J type. Usually abortion starts at the time the pits begin to harden, reaches a maximum within the first week, and soon trails away to a very low level. Gumming starts about 11 days later and parallels the occurrence of abortion.

Sometimes, however, the frequency distributions of the lengths of aborted embryos are badly skewed as in the present example or are very flat and perhaps bimodal as for the University Farm data for the season 1939.[4] In such cases, $p(t)$ is two-humped and very difficult to represent adequately by the first four moments, although a fairly good notion of $p(t)$ can be obtained by trial and error. The $p(t)$ for Sutter County, 1939, shows a very high incidence of abortion at the time of pit hardening and a small secondary peak of abortions about 6 weeks later. This second peak is not associated with pit hardening in point of time and should be investigated. It is not known whether this second peak in abortions is reflected in the occurrence of gumming. In the University Farm 1939 data the secondary peak must be much more important than in the Sutter County 1939 data.

It is regretted that frequency distributions of lengths of normal embryos at intervals throughout the season, the frequency distribution of the lengths of aborted embryos at harvesttime, and the probability distribution of gumming are not all available for the same orchard in the same season. Since such data are not available the above statements must be considered as only tentative and subject to revision on the accumulation of more data.

It is suggested that the functions of $m(t)$, $\sigma(t)$, and $\alpha(t)$ be more carefully determined by shortening the interval of time between observed frequency distributions of normal embryos and that the possibility of a major cause of embryo abortion late in some seasons be investigated.

SUMMARY

A mathematical model of embryo abortion in Phillips Cling peaches has been constructed. Observed facts seem to indicate the efficacy of the proposed model. The probability distribution of abortion may be one-humped or two-humped. One hump is always associated with pit hardening. The gumming of Phillips Cling starts about 11 days after pit hardening and hence occurs about 11 days after abortion begins. The probability of gumming parallels the probability of abortion at least when $p(t)$ is one-humped and perhaps so or nearly so when $p(t)$ is two-humped.

[4] See footnote 3, p. 175.

ROOT ROT OF RANUNCULUS ASIATICUS CAUSED BY PYTHIUM DEBARYANUM [1]

By C. M. TOMPKINS, *assistant plant pathologist*, and JOHN T. MIDDLETON, *junior plant pathologist, California Agricultural Experiment Station* [2]

INTRODUCTION

A destructive root disease of Persian buttercup (*Ranunculus asiaticus* L.) was first observed in commercial field plantings at Inglewood and Pacific Palisades, Calif., during the winter of 1937–38. Subsequently the disease has also been found in Santa Cruz, San Mateo, and San Francisco Counties. The losses are reflected not only in decreased field stands of plants, but in a smaller cut-flower crop and yield of tubers. Because of its economic importance, an investigation of this disease was conducted during the past four seasons, the results of which are briefly recorded in this paper. A preliminary note has previously been published.[3]

SYMPTOMS OF THE DISEASE

Ranunculus plants, whether produced from seeds or tubers, appear to be highly susceptible to root rot infection when grown in the field. The most striking symptoms of the disease consist of a general wilting followed by rapid collapse and death of the plant. The roots, tubers, stems, and petioles may be invaded. The roots and tubers of infected plants are dark brown, water-soaked, and flaccid. The stem plate is discolored and dark, but usually not water-soaked or flaccid. Occasionally the petioles become infected, in which event the diseased parts are dark brown, frequently exhibiting necrotic streaks which are 1 to 3 mm. wide and about 30 to 60 mm. long, extending outward from the base of the petiole and parallel to its long axis. In the field, plants are most commonly affected when 6 to 8 inches high, but, under experimental conditions, plants of all ages appear to be highly susceptible. The disease occurs on widely different soil types, ranging from coarse sand to heavy clay, and is favored by excessive rainfall or irrigation, poor drainage, cool weather, and crowding of plants.

THE CAUSAL FUNGUS, *PYTHIUM DEBARYANUM*

Isolations made from diseased material on water or malt-extract agars have consistently yielded a fungus which has been identified as *Pythium debaryanum* Hesse. The four isolates of the fungus studied, representing two districts in southern and two in central California where the disease occurs, are readily cultivated on potato-

[1] Received for publication May 13, 1941.
[2] The assistance of nontechnical employees of the Federal Work Projects Administration is acknowledged. Project No. 65-1-08-91.
[3] TOMPKINS, C. M. and MIDDLETON, J. T. ROOT ROT OF RANUNCULUS ASIATICUS CAUSED BY PYTHIUM DEBARYANUM. (Abstract) Phytopathology 29: 828. 1939.

dextrose, malt, corn-meal, and other agars, on which they develop a copious, dense, aerial mycelium. The asexual and sexual reproductive organs are sparingly produced in the surface growth but develop rather profusely intramatrically. Both types of fruiting bodies are abundant in the diseased tissues of tubers of infected plants. Perhaps the best medium for observing the development of the reproductive structures is plain water agar. On this substrate, the sporangia are usually spherical, thin and smooth-walled, and acrogenous; numerous intercalary sporangia are produced which may be spherical or barrel-shaped. Sporangia germinate either by the production of zoospores in a vesicle or by several germ tubes. Oogonia are similar to sporangia and have an average diameter of 22.2μ. Antheridia are usually plural, varying in number from one to six,

FIGURE 1.—Root rot of Persian buttercup: *A*, Symptoms produced on young Persian buttercup plant by *Pythium debaryanum* 12 days after inoculation in the greenhouse; *B*, noninoculated control.

predominantly two to three per oogonium, monoclinous and diclinous, when monoclinous arising some distance below the oogonium and not adjacent to it. One fertilization tube is usually produced by each antheridium applied to the oogonial wall. The oospores are spherical and smooth-walled, with a mean diameter of 18.2μ. Germination of oospores has not been observed.

The temperature-growth relations of the fungus have been determined for two of the four isolates. They are: Minimum, 1° C., optimum, 28°, and maximum 37°.

All four isolates of the fungus proved pathogenic to healthy ranunculus plants grown from seeds and tubers. Inoculum was prepared by growing the fungus on sterilized cracked wheat in 8-inch test tubes. When ready for use, this was added to autoclaved soil contained in 6-inch pots in such a manner as to avoid injuring the root system of the young plants. Sterile cracked wheat was added to the pots serving as controls. All pots were heavily watered each day in order to keep the soil very moist. The incubation period

ranged from 11 to 25 days, but all infected plants died within a day or two after the foliage commenced to wilt (fig. 1). Of 20 plants inoculated with each isolate, none escaped infection, while the 20 control plants remained healthy. The fungus was reisolated from each infected plant and proved to be identical with the original isolates. When tested in parallel series, the reisolates again proved highly pathogenic. In these tests, the infected plants exhibited symptoms identical with those of naturally infected plants. The appearance of the root system of artificially infected plants, as contrasted with healthy roots, is shown in figure 2.

Although *Pythium ultimum* Trow and *P. irregulare* Buis. are commonly associated with root rots of various herbaceous ornamentals

FIGURE 2.—Root rot of Persian buttercup: *A*, *B*, *C*, Symptoms produced on the roots of young Persian buttercup plants by *Pythium debaryanum* 12 days after inoculation in the greenhouse; *D*, noninoculated control.

in California, they have not been found associated with this disease. Inoculation experiments have shown that *P. ultimum* is strongly pathogenic to *Ranunculus* while *P. irregulare* is weakly pathogenic but likewise capable of inducing disease. The symptoms produced by these two species are similar to those produced by *P. debaryanum*.

EXPERIMENTAL HOST RANGE

In studies on the host range, one isolate of the fungus from southern California was used along with the no-wound technique described for the pathogenicity tests. Healthy plants to be inoculated had four to six leaves. Reisolations were made from all experimentally infected plants, and the reisolate of the fungus from a particular host was then tested by inoculation into healthy specimens of that host.

Since it is generally believed that *Pythium debaryanum* is cosmopolitan in its attacks, a large variety of plants including many of those grown in rotation with *Ranunculus* were experimentally inoculated. The experimental host range proved to be very limited and includes Iceland poppy (*Papaver nudicaule* L.), columbine (*Aquilegia caerulea* James), fibrous-rooted begonia (*Begonia semperflorens* Link and Otto), butterflyflower (*Schizanthus pinnatus* Ruiz and Pav.), and cucumber (*Cucumis sativus* L.). If seeds had been sown in experimentally infested soil the host range might have been extended. If this technique were employed the results would be indicative of the ability of *P. debaryanum* to cause damping-off and would not indicate its potentialities as a root rotting agent of more mature plants. Infection of these hosts, characterized by sudden wilting without change in color of the foliage and water-soaked lesions on the fibrous roots, occurred in 11, 18, 27, 9, and 17 days, respectively.

No infection was obtained in 41 species of plants representing 37 genera in 23 families, as follows:

Aizoaceae:
- Iceplant (*Mesembryanthemum crystallinum* L.)

Amaranthaceae:
- Cockscomb (*Celosia argentea* L. var. *cristata* Kuntze)

Campanulaceae:
- Canterbury-bells (*Campanula medium* L.)

Caryophyllaceae:
- Sweet-william (*Dianthus barbatus* L.)
- Carnation (*Dianthus caryophyllus* L.)

Chenopodiaceae:
- Spinach (*Spinacia oleracea* L.) var. Bloomsdale

Compositae:
- Transvaal daisy (*Gerbera jamesonii* Hook. var. *transvaalensis* Hort.)
- China-aster (*Callistephus chinensis* Nees) var. Giant Branching White, wilt resistant
- French marigold (*Tagetes patula* L.)
- African marigold (*Tagetes erecta* L.)
- *Gaillardia pulchella* Foug. var. *picta* Gray
- Hybrid cineraria (*Senecio cruentus* DC.)

Cruciferae:
- Cabbage (*Brassica oleracea* L. var. *capitata* L.) var. Winter Colma
- Cauliflower (*Brassica oleracea* var. *botrytis* L.) var. February
- Radish (*Raphanus sativus* L.) var. White Icicle
- Dames violet (*Hesperis matronalis* L.)
- Annual stock (*Matthiola incana* R. Br. var. *annua* Voss) var. Fiery Blood Red

Dipsacaceae:
- Mourning bride or pincushion flower (*Scabiosa atropurpurea* L.)

Labiatae:
- Flowering sage (*Salvia farinacea* Benth.)

Leguminosae:
- Broadbean (*Vicia faba* L.)

Malvaceae:
- Hollyhock (*Althaea rosea* Cav.)

Onagraceae:
- *Godetia grandiflora* Lindl.

Papaveraceae:
- California poppy (*Eschscholtzia californica* Cham.)
- Oriental poppy (*Papaver orientale* L.)

Polemoniaceae:
- *Phlox drummondii* Hook.

Ranunculaceae:
- Poppy anemone (*Anemone coronaria* L.)
- Love-in-a-mist (*Nigella damascena* L.)
- Rocket larkspur (*Delphinium ajacis* L.)

Ranunculaceae (*continued*):
Hybrid delphinium (*Delphinium cultorum* Voss)
Meadowrue (*Thalictrum dipterocarpum* Franch.)
Resedaceae:
Mignonette (*Reseda odorata* L.)
Rosaceae:
Geum chiloense Balb.
Scrophulariaceae:
Foxglove (*Digitalis purpurea* L.)
Snapdragon (*Antirrhinum majus* L.)
Pentstemon or beardtongue (*Pentstemon barbatus* Nutt.)
Solanaceae:
Tomato (*Lycopersicum esculentum* Mill. var. *vulgare* Bailey) var. Early Santa Clara Canner
Tobacco (*Nicotiana tabacum* L.) var. Turkish
Tropaeolaceae:
Garden nasturtium (*Tropaeolum majus* L.)
Umbelliferae:
Celery (*Apium graveolens* L.) var. Golden Self Blanching
Verbenaceae:
Garden verbena (*Verbena hybrida* Voss)
Violaceae:
Pansy (*Viola tricolor* L.)

DISCUSSION

In California, Persian buttercup plants are grown commercially in the field from seeds and tubers for seed, tuber, and cut-flower production. When grown for cut flowers, growers generally prefer to plant seeds, which are usually drilled in rows in August. The young plants, which commence to flower early in January if not infected, are frequently invaded by the fungus in the December preceding, or soon after the winter rains commence. When grown on poorly drained land, the mortality may reach 50 percent or more. Since limited tests of various strains of seeds and tubers from different sources failed to disclose any evidence of resistance to the disease, control is dependent upon the selection of well-drained sites.

SUMMARY

A destructive root disease of Persian buttercup (*Ranunculus asiaticus*) is described.

The disease is prevalent in commercial plantings in four coastal counties of California. It is favored in its development and spread by excessive moisture, poor drainage, cool weather, and crowding of plants.

The chief symptoms of the disease consist of a general wilting followed by rapid collapse and death of the plant. The roots, tubers, stems, and petioles may be affected.

Pythium debaryanum has been consistently isolated from infected plants and has proved pathogenic in greenhouse tests. The morphology and temperature relations of the fungus are discussed.

The incubation period for the disease ranges from 11 to 25 days.

In greenhouse tests, the fungus proved pathogenic to young plants of Iceland poppy, columbine, fibrous-rooted begonia, butterflyflower, and cucumber.

Persian buttercup seedlings were also experimentally infected with *Pythium ultimum* and *P. irregulare*. The symptoms were similar to those produced by *P. debaryanum*.

PANTOTHENIC ACID IN THE NUTRITION OF THE PIG[1]

By E. H. HUGHES[2]
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INTRODUCTION

As early as 1938, Hughes [3] reported that the addition of a rice bran filtrate to a purified diet containing thiamin, riboflavin, and nicotinic acid resulted in more rapid and economical growth in the pig. This product was a good source of what was then known as the filtrate factor. In the spring of 1940 sufficient quantities of synthetic optically inactive pantothenic acid were available to determine whether this substance was necessary for growth and physical well-being in the pig.[4]

EXPERIMENTAL DATA

Two experiments were conducted. The experimental procedure, equipment, and pigs used were similar to those employed in studies formerly reported [5] on the factors of the vitamin-B complex. The basal diet and salt mixture were similar to, but not identical with, those used previously. The basal ration consisted of beet sugar 81 percent, casein (purified) 15, salt mixture 4, cod-liver oil (Squibb's tested) 10 cc. per pig weekly, thiamin 3 mg., riboflavin 3 mg., nicotinic acid 15 mg., and vitamin B_6 5 mg. per 100 pounds of pig daily. In these diets where synthetic pantothenic acid (calcium pantothenate) was included it was fed at a level of 14.7 mg. per 100 pounds of pig daily, or 6.8 mg. of pantothenic acid.

The salt mixture was composed of the following salts: Calcium phosphate (dibasic) 40 percent, sodium chloride 20, calcium carbonate 11, ferric chloride 3, copper sulfate 0.2, manganese sulfate (anhydrous) 0.5, zinc oxide 0.1, magnesium citrate 15, cobaltous acetate 0.1, potassium chloride 10, and potassium iodide 0.1

In the first experiment, which was begun September 24, 1940, and continued until November 19, 1940, only five pigs were available. Two pigs were fed the basal diet without pantothenic acid, and three pigs were fed the same diet to which 6.8 mg. of pantothenic acid was added per 100 pounds of pig daily. Growth of these two groups is presented graphically in figure 1, *A*.

The pigs in group 1 grew very slowly while those in group 2 gained an average of 0.82 pound per head daily, a very good gain for pigs of their weight and age.

[1] Received for publication May 8, 1941. The experimental work reported in this paper became cooperative with the Bureau of Animal Industry, U. S. Department of Agriculture, July 1, 1938.

[2] The author takes pleasure in acknowledging the fine cooperation and assistance of N. R. Ittner and Robert Squibb, both of this station, for feeding, caring for, and photographing the animals used in this study.

[3] HUGHES, E. H. THE VITAMIN-B COMPLEX AS RELATED TO GROWTH AND METABOLISM IN THE PIG. Hilgardia 11: 595–612, illus. 1938.

[4] Pantothenic acid as calcium pantothenate was made available through the courtesy of Dr. S. H. Babcock.

[5] HUGHES, E. H. THE ROLE OF RIBOFLAVIN AND OTHER FACTORS OF THE VITAMIN-B COMPLEX IN THE NUTRITION OF THE PIG. Jour. Nutr. 17: 527–533, illus. 1939.

——— THE MINIMUM REQUIREMENT OF RIBOFLAVIN FOR THE GROWING PIG. Jour. Nutr. 20: 233–238, illus. 1940.

——— THE MINIMUM REQUIREMENT OF THIAMIN FOR THE GROWING PIG. Jour. Nutr. 20: 239–241, illus. 1940. (See also reference cited in footnote 3.)

The second experiment covered a period of 84 days, beginning November 26, 1940, and ending February 18, 1941. There were 10 pigs in this test, 5 in each group. An examination of the growth curves in figure 1, *B*, shows that the growth of pigs in this experiment was similar to that in the first experiment. The pigs in group 1 grew slowly, but those in group 2, fed pantothenic acid, made an average daily gain of 0.74 pound.

Among the outward manifestations of a deficiency of pantothenic acid in these studies were an early decrease in appetite and slow growth of the pigs, and an inability to move about in a normal manner within about a month after being placed on a deficient diet. The pigs apparently lost their sense of equilibrium and correlation; they "goose-stepped" and often fell; they became thin and emaciated; their hair became dry and finally after a period of about 70 days two of the pigs lost most of their hair; the feces were often watery, and in some instances blood was passed. The photographs (fig. 2) supplement the description given.

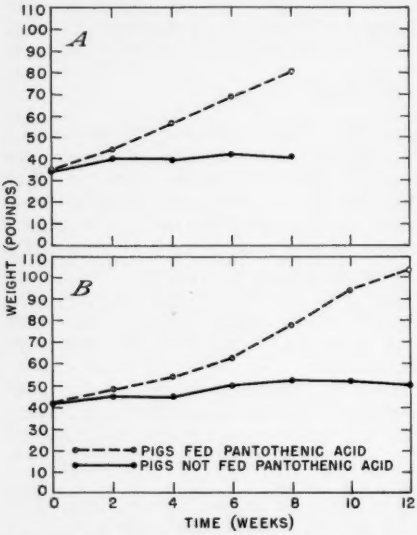

FIGURE 1.—Growth curves of pigs fed a basal diet deficient in pantothenic acid as compared with those of pigs fed 6.8 mg. of pantothenic acid per 100 pounds of body weight daily: *A*, experiment 1; *B*, experiment 2.

Four of the pigs were autopsied. One died on the seventy-seventh day and another soon after the second experiment was concluded; the other two were killed. The autopsies showed gastritis in each, which included a reddened area on the floor of the stomach about the size of a normal hand. Scattered throughout this area were hemorrhagic spots ranging in size from 1 to 2 mm. There was some inflammation in the large intestine of each, and in one there were many abscesses (fig. 2, *D*). On the skin of the two pigs that lost most of their hair, there were many reddened areas about 7 mm. in diameter.

DISCUSSION

Chick et al.[6] and Hughes suggest that pigs fed diets sufficient in nicotinic acid, thiamin, and riboflavin need other factors of the vitamin-B complex. Further evidence of the necessity for one or both of factors 1 (vitamin B_6) and 2 (the filtrate factor) was pre-

[6] CHICK, HARRIETTE, MACRAE, THOMAS FOTHERINGHAM, MARTIN, ARCHER JOHN PORTER, and MARTIN, CHARLES JAMES. THE WATER-SOLUBLE B-VITAMINS OTHER THAN ANEURIN (VITAMIN B_1), RIBOFLAVIN AND NICOTINIC ACID REQUIRED BY THE PIG. Biochem. Jour. 32: 2207-2224, illus. 1938. (See also reference cited in footnote 3, p. 185.)

sented by Hughes.[7] In a recent publication Wintrobe et al.[8] described symptoms in pigs which seem similar to those observed in this investigation in pigs deficient in pantothenic acid.

Goose-stepping in pigs has been reported from many sections of the country. It has been noticed at this station among pigs fed a heated diet composed largely of corn, wheat middlings, and casein (unpublished

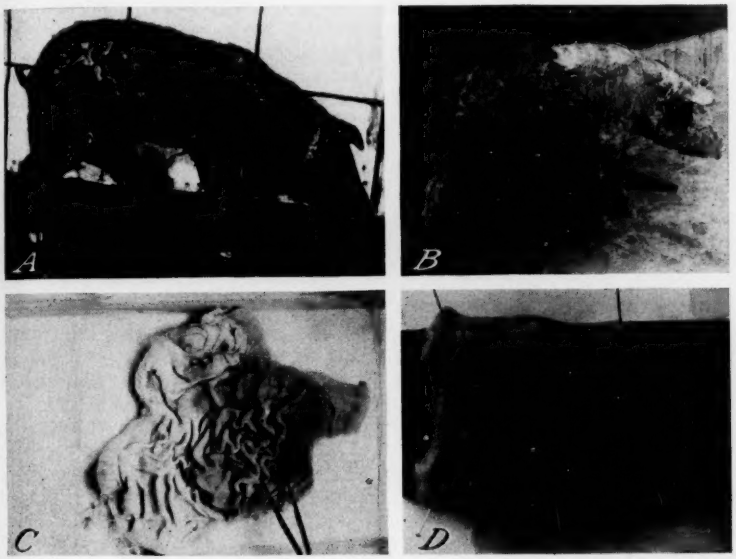

FIGURE 2.—Results of feeding pigs a basal diet deficient in pantothenic acid: *A*, Goose-stepping; *B*, loss of hair; *C*, stomach with reddened area indicating gastritis; *D*, abscesses in the large intestine. The matted condition of the hair was caused by the dirt sticking to it.

data) and among pigs fed a diet deficient in factor 2 (filtrate factor)[7]. Such pigs walked poorly and several became crooked in their hocks. This condition is associated with a deficiency of pantothenic acid.

SUMMARY

Young pigs fed a diet deficient in pantothenic acid developed subnormal appetites, grew slowly, became thin and emaciated, showed a lack of normal coordination, "goose-stepped," some lost their hair, and at autopsy showed gastritis and involvement of the large intestine. It is therefore concluded that pantothenic acid is necessary for normal growth and well-being in the young growing pig.

[7] See first reference footnote 5, p. 185.

[8] WINTROBE, MAXWELL M., MILLER, JOSEPH L., JR., and LISCO, HERMANN. THE RELATION OF DIET TO THE OCCURRENCE OF ATAXIA AND DEGENERATION IN THE NERVOUS SYSTEM OF PIGS. Johns Hopkins Hosp. Bul. 67: 377-404, illus. 1940.

THE POTASSIUM REQUIREMENT OF GROWING PIGS[1]

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INTRODUCTION

This paper reports the results of one of a series of studies of purified diets for pigs now being carried on at the California Experiment Station at Davis. During the summer of 1940 considerable difficulty was experienced with the purified diets that were being fed except the control diet in which skim-milk powder was included. In previous experiments where a rice-bran filtrate was fed at a 5-percent level no serious difficulties were encountered. One of the diets fed was cane sugar 82 percent, purified casein 15 percent, salt mixture 3 percent, thiamin 3 mg., riboflavin 3 mg., calcium pantothenate 14.7 mg. (pantothenic acid 6.8 mg.), nicotinic acid 15 mg., and vitamin B_6 5 mg. per 100 pounds of pig daily, and 10 cc. of cod-liver oil per pig weekly. The salt mixture had been used previously. On this ration the pigs soon lost their appetites and ceased to grow. They scoured to some extent, certain individuals vomited, others seemed weak in their hindquarters, and one was unable to rise. Similar pigs fed a diet of cane sugar 68 percent, purified casein 5 percent, skim-milk powder 25 percent, salt mixture 2 percent, and 10 cc. of cod-liver oil per pig weekly gained over 40 pounds in 4 weeks and seemed perfectly normal.

It was evident that some deficiency was responsible for the trouble. From the information at hand, it appeared that whatever was lacking was water-soluble, for when a rice-bran filtrate was fed at a 5-percent level the results were good. Skim-milk powder fed at a 25-percent level also provided something that was necessary. Since casein was present in the basal diet, it was expected that the necessary nutrient would be present in whey. At the time that these diets were being fed to pigs, the same diets were being fed to rats. Whey was accordingly fractionated and fed to growing rats, with the result that when the inorganic salts of whey were added to the deficient diet a decided improvement was noticed during the first week. When the salt mixture that was being used was compared to the inorganic salts in milk it was found that the potassium content of the salt mixture was probably much too low. Potassium chloride therefore was substituted in the rat diets for the inorganic salts of milk, and the results in growth were the same as with the inorganic salts of milk.

In the meantime, the pigs were in such a bad state of health that it appeared they would die. Whey powder was added to the diet and a decided improvement took place both in their appetites and in their growth. Later potassium was substituted for the whey powder with equally good results. With the facts established that the salt mixture which had been used was not adequate because of its lack of potassium and that potassium was necessary for the pig, the next step was to determine the minimum potassium requirement of the pig.

[1] Received for publication May 8, 1941. The experimental work reported in this paper became cooperative with the U. S. Department of Agriculture, Bureau of Animal Industry, July 1, 1938.

MATERIAL AND METHODS

Four groups of five pigs each, uniform in size and weighing about 50 pounds at the beginning of the experiment, were used in this study. They were fed and watered in steel troughs. Their quarters were similar in size and shape and had concrete floors and wooden partitions. The wooden partitions were covered with small-mesh wire to keep the pigs from chewing the boards. The pigs had access to inside and outside pens.

The basal diet consisted of beet sugar 81 percent, purified casein 15 percent, salt mixture 4 percent, cod-liver oil 10 cc. per pig weekly, thiamin 3 mg., riboflavin 3 mg., nicotinic acid 15 mg., pantothenic acid 6.8 mg., and vitamin B_6 5 mg. per 100 pounds of pig daily. The salt mixture was composed of calcium phosphate (dibasic) 45 percent,

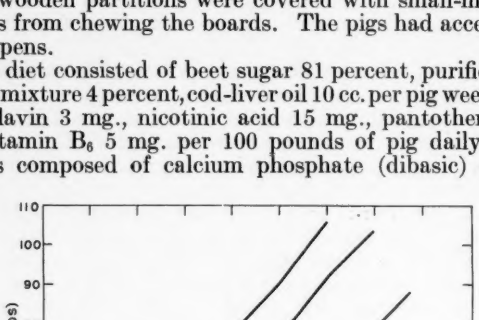

FIGURE 1.—The effect of the level of potassium intake upon the growth of pigs: Lot 1 received no potassium; lots 2, 3, and 4 received 2.25, 4.5, and 9.5 gm. of potassium chloride per 100 pounds of body weight daily.

sodium chloride 22 percent, calcium carbonate 12 percent, ferric chloride 3 percent, magnesium citrate 17 percent, cobaltous acetate 0.1 percent, copper sulfate 0.2 percent, manganese sulfate (anhydrous) 0.5 percent, zinc oxide 0.1 percent, potassium iodide 0.1 percent.

The following diets were fed, beginning November 26, 1940:

Lot 1.—Basal (no potassium except the potassium iodide in the salt mixture).
Lot 2.—Basal+2.25 gm. of potassium chloride per 100 pounds of pig per day.
Lot 3.—Basal+4.5 gm. of potassium chloride per 100 pounds of pig per day.
Lot 4.—Basal+9.5 gm. of potassium chloride per 100 pounds of pig per day.

The sugar, casein, and salt mixture were weighed and mixed thoroughly at the beginning of the experiment. The vitamins were weighed weekly, put in water solution, and mixed with the feeds for the various lots. The amount of gain and the feed consumption of the previous week made it possible to determine the amounts that would be consumed the following week. The potassium was weighed weekly and handled like the vitamins. The feed mixtures were kept in paper-lined garbage cans, the feed for each lot in a different one. Separate buckets were used to feed each lot. The pigs were weighed individually.

EXPERIMENTAL DATA

The growth rate of the four lots is presented graphically in figure 1. The pigs in lot 1 gained slightly for a time then lost weight. Those in lot 2 gained throughout the feeding period of 12 weeks, though not so rapidly as those in lots 3 and 4. One pig in lot 2 gained slightly at first, then lost weight and finally died. The pigs in lots 3 and 4 gained at practically the same rate. The gains of all lots were slow at first because of the change from a normal grain diet to a highly purified diet and because of the relatively small daily food consumption of small pigs.

At the end of 4 weeks all the pigs in lot 1 had to be helped to rise. Three had died. The first indication of a deficiency was reduced appetite; soon thereafter the pigs would stand and squeal and become unsteady on their legs. Later they would be found on the floor and would be unable to get up without help. Once on their feet they could move around reasonably well and were able to fight for their feed at the trough. Two of the pigs lived throughout the experiment. However, they became very thin and emaciated, their appetites were poor, and they remained unsteady on their legs.

At autopsy the three that died early were found to be in fairly good condition and they were not thin. In each case there was feed in the stomach with little or no feed or feces in the small or large intestine. In one there was a large congested area on the wall of the stomach. In the other two there were ulcers in the stomach, some small, others measuring about 15 mm. in diameter, and the large intestine showed inflammation and some hemorrhagic areas. The two pigs that remained on the experiment were killed at its conclusion and autopsied. They were exceedingly thin and emaciated and showed some inflammation of the walls of the stomach. It would appear, therefore, that diets deficient in potassium affect the mucous membrane of the stomach and large intestine.

DISCUSSION

In this study a highly purified diet was fed to growing pigs with and without sufficient potassium. Other workers have reported the requirements of this element for the rat and the chick. Osborne and Mendel [2] found the growth of rats to be fairly satisfactory on a potassium intake of about 0.03 percent provided the sodium intake was adequate. Miller [3] stated that the growth of rats can be greatly retarded by reducing the potassium content below approximately 0.1 percent. He found too that substituting sodium for potassium failed to produce normal growth in rats.

Heppel and Schmidt [4] reported a negative balance when lactating mothers (rats) were fed a potassium level of 0.14 percent in the diet.

Recently Ben Dor,[5] working with growing chicks, reported that at least 0.17 percent of potassium was required if approximately maximal growth was to be expected and that more than 0.13 percent was required to prevent heavy mortality.

[2] OSBORNE, THOMAS B., and MENDEL, LAFAYETTE B. THE INORGANIC ELEMENTS IN NUTRITION. Jour. Biol. Chem. 34: 131–139, illus. 1918.

[3] MILLER, H. G. POTASSIUM IN ANIMAL NUTRITION. II. POTASSIUM IN ITS RELATION TO THE GROWTH OF YOUNG RATS. Jour. Biol. Chem. 55: 61–78, illus. 1923.

[4] HEPPEL, LEON A., and SCHMIDT, CARL L. A. STUDIES ON THE POTASSIUM METABOLISM OF THE RAT DURING PREGNANCY, LACTATION, AND GROWTH. Calif. Univ. Pubs., Physiol. 8: 189–205, illus. 1938.

[5] BEN DOR, BEN-AMI. REQUIREMENT OF POTASSIUM BY THE CHICK. Soc. Expt. Biol. and Med. Proc. 46: 341–343. 1941.

The results reported here indicate that the requirement for growing pigs lies between 2.25 gm. (lot 2) and 4.5 gm. (lot 3) of potassium chloride, or in terms of potassium, 1.18 gm. and 2.36 gm. respectively, per 100 pounds of pig daily.

The growth rate of the pigs in lot 2 was considerably less than that of the pigs in lot 3, while the gains of lots 3 and 4 were similar. The additional potassium given to the pigs in lot 4 over that of lot 3 appeared to be unnecessary. These figures stated in terms of the diet fed are 0.08 percent and 0.15 percent. Inasmuch as the pigs in lot 2 gained less rapidly than those in lots 3 and 4 and one pig in this lot died, 1.18 gm. of potassium per 100 pounds of pig daily or 0.08 percent of the diet is not sufficient; therefore, the requirement is probably near 2.36 gm. of potassium per 100 pounds daily or 0.15 percent of the ration. These figures are in fair agreement of those of Miller, Heppel, and Schmidt for the rat and Ben Dor for the chick.

SUMMARY

It has been shown in these studies that potassium is necessary for normal growth in the young pig.

Under the conditions of this experiment the requirement of potassium chloride for the young pig lies between 2.25 gm. and 4.5 gm. per 100 pounds of pig daily. In terms of potassium this amounts to 1.18 gm. and 2.36 gm. respectively, and as percentage of the diet it amounts to 0.08 and 0.15 percent. Since 1.18 gm. of potassium per 100 pounds of pig daily or 0.08 percent of the diet was not sufficient, 2.36 gm. per hundredweight of pig daily or 0.15 percent of the diet is considered the minimum requirement for young growing pigs.

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